A Randomized Trial of Omega-3 Dietary Supplementation, Anti-*Demodex* Eyelid Cleanser, and Standard Eyelid Cleanser for Pediatric Chalazia

Protocol Identifying Number: CH01
IND Sponsor: Jaeb Center for Health Research
IND #: 178499
NCT07496515

Funded by:
National Eye Institute, National Institutes of Health

Version Number: v1.1

21 July 2026

KEY ROLES

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VERSION HISTORY

VERSION NUMBER	AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION
1.0	Danielle Chandler	Raymond Kraker	21Apr2026	Original version
1.1*	Danielle Chandler	Raymond Kraker	21Jul2026	• Changed enrollment exam photographs to be taken by the parent rather than the investigator. As such, amended eligibility criteria to require that the parent's smartphone or other digital device that can take photographs must be present at the enrollment visit. Also deleted home parental photos taken within a day after enrollment. • Specified that parental photographs taken at the enrollment exam would be reviewed for all 8-week outcome assessments, including those made by the investigator and by the central reader. • Specified that the primary outcome is based on the 8-week in-office clinical exam but also the enrollment exam and the parental photographs taken at enrollment. • Eliminated eligibility criteria for working refrigerator given that omega-3 supplement does not require refrigeration. • Added ability to receive text or email during the study as inclusion criteria given the many reminders and requirement to complete survey during the study. • Added to statistical plan a tabulation of treatments prescribed at investigator discretion between 8 weeks and 12 months. • Removed section on adverse device effects as the Bruder mask is not considered an investigational device. • Clarified in visit grid that body weight is measured at enrollment as well as at 6 months. • Clarified in flow chart that medical history, including adverse events and concomitant medications are collected at each follow up visit. • Changed allowable window for 8-week primary office visit (and parental photographs) to 6 to 12 weeks post-randomization (rather than 4 to 12 weeks as previously stated. • Combined slit lamp, palpation, and grading of Demodex collarettes into "clinical exam" • Minor edits and clarifications throughout.

*Version in effect when first participant enrolled

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
ADHD	Attention Deficit Hyperactivity Disorder
AE	Adverse Event
ANCOVA	Analysis of covariance
CI	Confidence interval
CFR	Code of Federal Regulations
CRF	Case report form
DHA	Docosahexaenoic acid
DHHS	Department of Health and Human Services
DSMC	Data safety and monitoring committee
eCRF	Electronic case report form
EFAs	Essential fatty acids
e.g.,	For example,
EPA	Eicosapentaenoic acid
FDA	Food and Drug Administration
g	Gram
GABA	γ-aminobutyric acid
GCP	Good clinical practice
ICH	International Council for Harmonization
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Intention to treat
JCHR	Jaeb Center for Health Research
kg	Kilogram
LAR	Legally authorized representative
lbs	Pounds
mg	Milligram
mL	Milliliter
MOP	Manual of Procedures
MRD-1	Margin reflex distance 1
NIH	National Institutes of Health
NSAID	Non-steroidal anti-inflammatory drug
PEDIG	Pediatric Eye Disease Investigator Group
PI	Principal Investigator
PRN	Physician Recommended Nutraceuticals
QA	Quality assurance
QC	Quality control
RBM	Risk-based monitoring
RCT	Randomized clinical trial
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SUSAR	Suspected unexpected serious adverse reaction
US	United States

SITE PRINCIPAL INVESTIGATOR STATEMENT OF COMPLIANCE

Protocol Title: A Randomized Trial of Omega-3 Supplementation, Anti-*Demodex* Eyelid Cleanser, and Standard Eyelid Cleanser for Pediatric Chalazia

Protocol Version: v1.1 21Jul2026

Each clinical site approved to participate in the study will have one individual designated as the Protocol Principal Investigator (Protocol PI) at the site for that protocol. This may or may not be the same investigator who serves as the overall Site Principal Investigator (Site PI) for all PEDIG studies.

As Protocol PI, I have read the protocol specified above. In my formal capacity as Protocol PI, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the Jaeb Center for Health Research, which serves as the Coordinating Center for the protocol, with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site.

This trial will be carried out in accordance with ICH E6 Good Clinical Practice (GCP) and as required by the following: United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312).

As the Principal Investigator, I will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), or other approved Ethics Committee, except where necessary to eliminate an immediate hazard(s) to the trial participants.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Participants Protection Training and Good Clinical Practice Training. Further, I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator's Signature _____ Date: _____ / _____ / _____
dd mmm yyyy

Principal Investigator's Name: _____

Site Number: _____

PROTOCOL SUMMARY

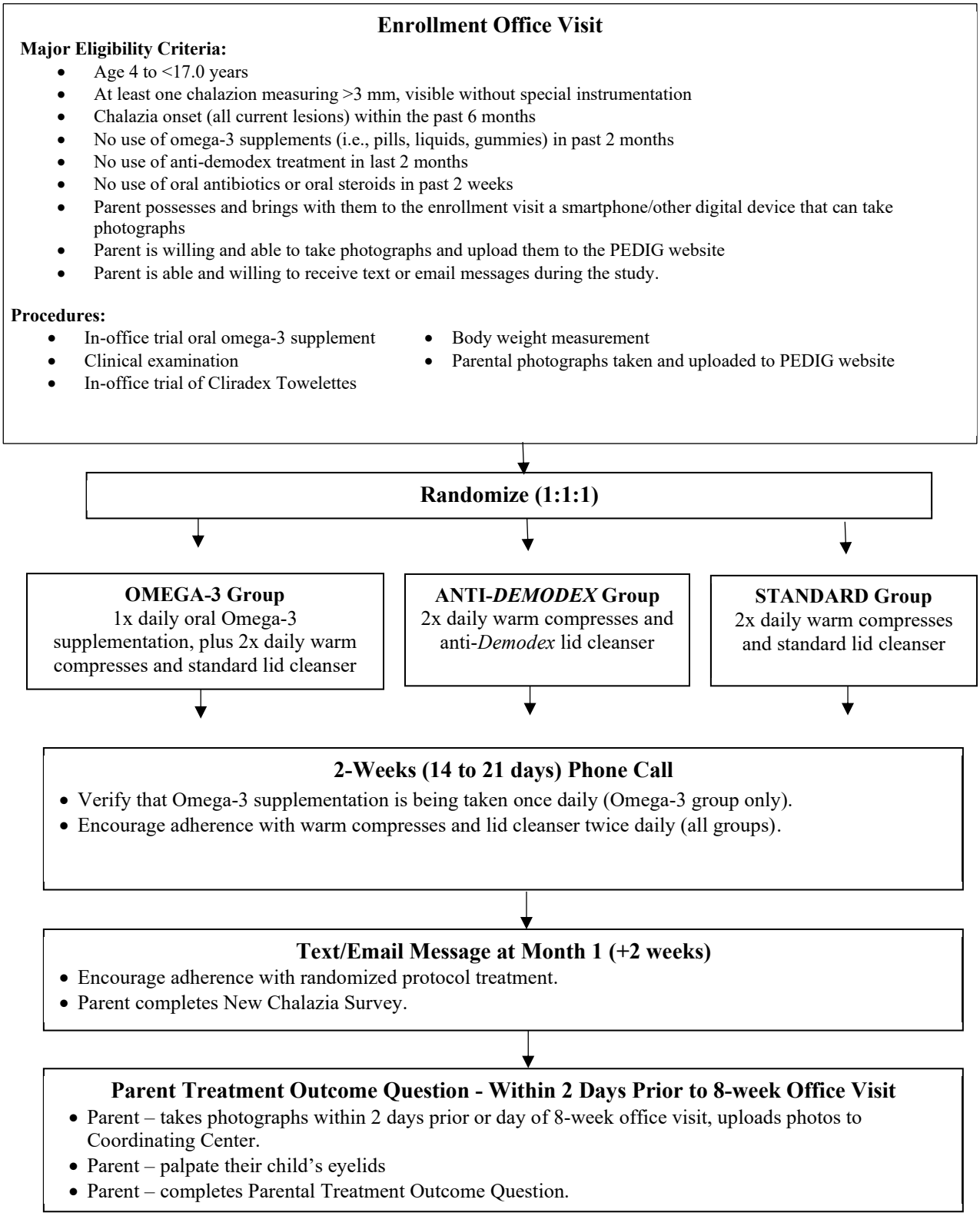
PARTICIPANT AREA	DESCRIPTION
Title	A Randomized Trial of Omega-3 Supplementation, Anti-*Demodex* eyelid Cleanser, and Standard eyelid Cleanser for Pediatric Chalazia
Précis	The RCT will address two primary questions: 1) Is treatment with anti-*Demodex* eyelid cleanser superior to standard eyelid cleanser for improvement or resolution of chalazia at 8 weeks? 2) Is treatment with omega-3 supplementation for one year superior to no omega-3 supplementation for one year for the prevention of new chalazia between 8 weeks and 12 months? In addition, parents will take standardized photographs of their children's eyelids twice, at the enrollment visit and within two days prior to or on the day of the 8-week visit. For 8-week outcome assessment, photographs will be supplemented with a Parental Treatment Outcome Question and parental palpation of the eyelids before the 8-week visit. The objective is to evaluate the agreement between a masked central reader's review of 8-week telehealth information (8-week parental photos and Parental Treatment Outcome Question) and an in-office clinical assessment by a masked investigator ["gold standard"]) when determining whether chalazia have improved/resolved (vs. not) at 8 weeks, across all treatment groups. Treatment with warm compresses and one of two different eyelid cleansers is mandated in each group for the first 8 weeks of the study. Treatment between 8 weeks and 12 months is at investigator discretion; however, the omega-3 group must continue omega-3 supplementation through 12 months, and the anti-*Demodex* eyelid cleanser and standard eyelid cleanser groups must NOT receive any omega-3 supplementation. Development of new chalazia between 8 weeks and 12 months will be assessed at 6- and 12-month follow-up visits, as well as by querying parents with monthly text or email message reminders and monthly online surveys. "New chalazion" is defined as a new lesion developing in the same location as a previously resolved chalazion or in a new area on any eyelid.
Investigational Drugs	Physician Recommended Nutraceuticals (PRN)® Eye Omega Benefits for Kids Cliradex® Towelettes OCuSOFT® Eyelid Scrub Plus Pre-Moistened Pads
Treatment Groups	Eligible participants will be randomly assigned 1:1:1 to one of three treatment groups: • Oral omega-3 supplementation (eicosapentaenoic acid and docosahexaenoic acid) – once daily dose of 5 mL (1000mg) or 10 mL (2000 mg total), depending on age and weight, for 12 months and twice daily warm compresses (using a Bruder® Mask) and standard eyelid cleanser (OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads) for 8 weeks. Omega-3 supplementation will continue until the 12-month outcome visit; otherwise, treatment is at investigator discretion after 8 weeks (hereafter **OMEGA-3** group). • Twice daily warm compresses and anti-*Demodex eyelid* cleanser (Cliradex® Towelettes) for 8 weeks. No Omega-3 supplementation can be used during the entire 12-months; otherwise, treatment is at investigator discretion after 8 weeks (hereafter **ANTI-*DEMODEX*** group). • Twice daily warm compresses and standard eyelid cleanser (OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads) for 8 weeks. No Omega-3 supplementation can be used for the entire 12-months; otherwise, treatment is at investigator discretion after 8 weeks (hereafter **STANDARD** group).

PARTICIPANT AREA	DESCRIPTION
Objectives	**Primary Objectives:** • To evaluate the efficacy of **ANTI-*DEMODEX*** versus **STANDARD** for improvement or resolution of chalazia at 8 weeks. • To evaluate the efficacy of **OMEGA-3** versus **STANDARD** for prevention of new-onset chalazia between 8 weeks and 12 months. **Telehealth Objective:** • To evaluate the agreement between an in-office clinical assessment by a masked investigator ("gold standard") versus a masked central reader's review of telehealth information (8-week parental photos, parental palpation of eyelids, and Parental Treatment Outcome Question) for determining whether chalazia have improved/resolved (vs. not) at 8 weeks, across all treatment groups. Secondary objectives mirror the primary objectives but 1) compare omega-3 to standard at 8 weeks and 2) compare anti-*Demodex* to standard between 8 weeks and 12 months.
Study Design	Multicenter Phase II Randomized Clinical Trial
Number of Sites	The study is open to all clinical sites approved to participate in the PEDIG network.
Endpoints	**Primary Efficacy Outcome for ANTI-DEMODEX vs. STANDARD:** • Proportion of participants whose chalazia are determined to be "improved or resolved" vs. "worse or unchanged" after 8 weeks, based on a masked investigator examination. **Primary Efficacy Outcome for OMEGA-3 vs. STANDARD:** • Proportion of participants with development of new chalazia between 8 weeks to 12 months based on all available data. **Key Secondary Telehealth Objective:** • To determine the agreement between a masked central reader's review of telehealth information (8-week parental photos, parental palpation of eyelids and Parental Treatment Outcome Question) versus an in-office clinical assessment by masked investigator ("gold standard") for improved/resolved chalazia outcome at 8 weeks, across all treatment groups. **Key Safety Outcomes:** • Proportion of adverse events reported. Secondary efficacy outcomes mirror the primary efficacy outcomes but 1) compare omega-3 to standard at 8 weeks and 2) compare anti-*Demodex* to standard between 8 weeks and 12 months.
Population	**Inclusion Criteria:** • Age 4 to <17 years. • At least one chalazion measuring >3 mm, visible without special instrumentation. • Chalazion onset (all current lesions) within the past 6 months by parental report. • Willing to forgo non-study use of omega-3 supplements (i.e., pills, liquid, gummies) for the duration of the study. • Prior or current treatment with warm compresses, eyelid cleanser/scrubs, topical antibiotic, and/or topical steroids IS allowed. • Topical antibiotics and topical steroids must be discontinued at study enrollment. • All non-study treatments for chalazia must be discontinued at enrollment for the first 8 weeks of the study. • Parent seeking treatment for child's chalazion/chalazia. • There is a working microwave oven in the home. • Parent possesses and brings to the enrollment visit a smartphone or other digital device that can take photographs. • Parent is able and willing to take photographs and upload them to the PEDIG website. • Parent is able and willing to receive text or email messages during study.

PARTICIPANT AREA	DESCRIPTION
	• The parent and participant are willing to comply with the assigned treatment regimen after an in-office trial of Cliradex® and Physician Recommended Nutraceuticals (PRN)® Eye Omega Benefits for Kids • Willing to consume fish-based oral supplements. • Contact lens correction of refractive error is allowed. **Exclusion Criteria:** • Current chalazion on any eyelid present for over 6 months by parental report. • Use of oral omega-3 supplements (i.e., pills, liquid, gummies) in the last 2 months. • Use of oral antibiotics or oral steroids within the prior 2 weeks. • Use of anti-*Demodex* treatment in the last 2 months. • Current or prior use of implanted steroids. • Prior surgery or injection for chalazia on any eyelid within the previous 6 months. • Presence of ptosis as defined by 2-mm or smaller marginal light-reflex distance 1 (MRD-1) (distance from corneal light reflex and bottom of upper eyelid). This includes ptosis covering the visual axis. Sectoral mechanical ptosis in the area of chalazia is allowed if the visual axis is not obscured, and the investigator does not deem the ptosis visually significant. • History of ptosis surgery. • Any conjunctivitis (e.g., phlyctenular keratoconjunctivitis, allergic, bacterial, and/or vernal) requiring treatment *other than* antihistamines, artificial tears, and/or cold compresses. • Intraocular inflammation of any kind within the last month. • Intraocular conditions that in the investigator's opinion may require use of oral or intraocular steroids, or systemic immunotherapy within the next year. • Current corneal erosion, infiltrates, ulcers, significant neovascularization (within 3 mm of optical center), or history of corneal graft or central corneal scarring • Known hypersensitivity or allergy to Cliradex® Towelette ingredients including terpinen-4-ol, a tea tree oil derivative. • Known hypersensitivity or allergy to OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads or its ingredients (Polyhexamethylene biguanide preservative (PHMB), provitamin B5, and decyl glucoside). • Known hypersensitivity or allergy to Bruder® mask. • Ocular rosacea. • Known hypersensitivity or allergy of fish or seafood. • Known hypersensitivity or allergy to oral omega-3 supplements. • Unable to consume liquid supplements orally. • Consumption of fish 4 or more times a week (on average). • Bleeding disorder or taking any anticoagulants/antiplatelet medications. • Daily use of non-steroidal anti-inflammatory (NSAID) or blood-pressure lowering medications. • Current chemotherapy treatment. • Current use of orlistat (Xenical or Alli). • Current use of any other medication that the investigator feels may significantly interact with omega-3 supplementation, not including oral contraceptives. • Diagnosis of any of the following conditions: ○ diabetes mellitus, heart rhythm disorder, underactive thyroid, liver disease or pancreatic disease • Moving outside the area in the next 12 months. • Other family member participating in the study. • Immediate family member (i.e., parent or legal guardian, sibling) is an investigator, coordinator, or certified tester directly affiliated with this study or an employee of the JAEB Center for Health Research. • Has previously been randomized as a participant in this study. • Developmental delays/sensory sensitivities that parents or the investigator feel would interfere with study treatment. • Pregnant, lactating or planning to become pregnant during study participation.

PARTICIPANT AREA	DESCRIPTION
Sample Size	A convenience sample of 168 (56 in each group) is expected to provide at least 150 participants (50 in each group) for analyses after adjusting for 10% loss to follow-up prior to the 12-month outcome.
Phase	Phase II Randomized Clinical Trial
Participant Duration	Participation in the study will end 12 months after enrollment.
Study Duration Planned	20 months from first enrollment until the last participant visit.
Protocol Overview/Synopsis	Participants enrolled into the study will be prescribed twice daily warm compresses for 8 weeks and randomly allocated (1:1:1) to receive either 1) Oral omega-3 supplementation and standard eyelid cleanser, 2) Anti-*Demodex* eyelid cleanser, or 3) Standard eyelid cleanser as treatment for chalazia. Participants will return for a visit after 8 weeks for a masked clinical exam to determine whether the chalazia has improved or resolved. As part of a telehealth objective, the 8-week visit will also include a masked central reader's assessment of telehealth data (8-week parent photographs, parental palpation of eyelids, and review of Parental Treatment Outcome Question) and enrollment data. After the 8-week visit, treatment is at investigator discretion other than 1) the **OMEGA-3** group must continue the study-prescribed Omega-3 supplementation and 2) the **ANTI-*DEMODEX*** and **STANDARD** groups must not receive Omega-3 supplementation. Participants will be followed from 8 weeks to 12 months from baseline to determine the proportion of participants with new chalazia based on monthly parental report (solicited via text/email messages), and office visits at 6 months and 12 months.

STUDY SUMMARY FLOW CHART



8-week (±2 weeks) Office Visit: Primary Outcome (Masked)

- Medical history (including adverse events and concomitant medications)
- Clinical exam
- Investigator completes 8-week Treatment Outcome Question (first time) based on 8-week in-office clinical examination, and review of enrollment clinical exam and parental photographs taken at enrollment.
- Investigator reviews 8-week telehealth information (8-week parental photos, parental palpation of eyelids, and Parental Treatment Outcome Question).
- Investigator completes 8-week Treatment Outcome Question (second time) based on review of telehealth information, enrollment clinical exam, parental photographs taken at enrollment. and 8-week in office clinical exam.
- Unmask investigator (masked examiner) to treatment group
- Treatment between the 8-week outcome exam and 12 months is at investigator discretion other than:
  - **Omega-3 group** must continue Omega-3 supplementation.
  - **Anti-*demodex* lid cleanser** and **Standard lid cleanser** groups must not receive Omega-3 supplementation.

Text/Email Messages at Months 3, 4, 5, 7, 8, 9, 10 and 11 (+2 weeks)

- Parent completes New Chalazia Survey, which includes taking photographs of any new chalazia that have developed.

6-month (±2 weeks) Office Visit (Unmasked)

- Medical history (including adverse events and concomitant medications)
- Clinical exam
- Investigator reviews Parental New Chalazia Survey results since last visit.

Treatment is at investigator discretion other than:

- **Omega-3 group** must continue Omega-3 supplementation
- **Anti-demodex lid cleanser** and **Standard lid cleanser** groups must not receive Omega-3 supplementation.

12-month (±2 weeks) Office Visit (Unmasked)

- Medical history (including adverse events and concomitant medications)
- Clinical exam
- Investigator reviews Parental New Chalazia Survey results since last visit.

SCHEDULE OF STUDY CALLS, VISITS, AND PROCEDURES

PROCEDURE	ENROLL-MENT VISIT	2-WEEK PHONE CALL (day 14- 21)	TEXT/EMAIL MESSAGES (1 month, 8-weeks, and months 3, 4, 5, 7, 8, 9, 10, and 11) (0 to +2 weeks)	8-WEEK PRIMARY OUTCOME OFFICE VISIT (±2 weeks)	6-MONTH OFFICE VISIT (±2 weeks)	12-MONTH OFFICE VISIT (±2 weeks)	CENTRAL READER TELE-HEALTH ASSESS-MENT
Consent/assent (Enrollment)	X						
Eligibility Assessment by Investigator	X						
Demographics	X						
Medical / Treatment History	X			X	X	X	
Body Weight Measurement	X				X		
Clinical Examination by Investigator	X			X masked	X unmasked	X unmasked	
Palpation of Eyelids by Parent				X[4]			
In-office Trial of Omega-3 Supplement	X						
In-office Trial of Cliradex® Eyelid Cleanser	X						
Parental Photographs of Eyelids	X[1]			X[4]			
Adverse Event Collection		X		X	X	X	
Randomized Treatment Adherence		X	1-month only	X	X	X	
Parental Treatment Outcome Question				X[4]			
Investigator Treatment Outcome Question[2]				X masked			
Central Reader Treatment Outcome Question[3]							CR
Parental New Chalazia Survey			X[5] except 1 month and 8 weeks		X[5]	X[5]	

[1] The initial set of parental photos should be taken at enrollment, as instructed by the investigator or coordinator.

[2] Treatment outcome question is assessed twice by the investigator at the 8-week visit:
1. based on the 8-week clinical exam and review of enrollment clinical exam only, and
2. based on the telehealth information (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question) and review of enrollment in-office exam, including site-taken photographs), review of enrollment clinical exam, and the 8-week clinical exam

[3]A masked central reader will asynchronously review the 8-week telehealth materials (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question) and enrollment in-office exam and parental photographs taken at enrollment. The masked central reader will then answer the (8-week) Investigator Treatment Outcome Question based on the telehealth data and enrollment data. This masked central reader assessment will be compared to the "gold standard" clinical exam for the telehealth objective. The masked central reader will be 1-2 investigators, each of whom cannot evaluate the telehealth data for participants at their own clinical site.

[4] The second set of parental photos, parental palpation of eyelids, and the Parental Treatment Outcome Question should be completed up to 2 days before the office visit or immediately prior to the 8-week office visit; they must occur before the investigator's evaluation at the 8-week office visit.

[5]Text/email messages will be sent at 1 month to encourage adherence with protocol treatment, at 8 weeks to remind the parent to complete home tasks within 2 days before the 8-week visit (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question), and at months 3, 4, 5, 7, 8 9, 10 and 11 for parent to complete Parental New Chalazia Survey. At 6- and 12-months, Investigator reviews Parental New Chalazia Survey results since last visit.

Chapter 1: Background Information

1.1 Clinical Characteristics and Epidemiology

A chalazion is a localized swelling in the eyelid caused by obstruction of the meibomian glands, followed by lipogranulomatous inflammation. Although not caused by bacteria, the site can become secondarily infected by bacteria. There is often no identified underlying cause for chalazia. However, several local risk factors have been identified, including *Demodex* infestation (in 53% to 73% of cases),[1-3] meibomian gland dysfunction,[4] blepharitis,[4] and ocular rosacea.[5, 6] Associated systemic factors include vitamin A deficiency[7] and low serum ferritin levels.[8] Differences in the meibum and lid margin microbiome between subjects with chalazia and healthy controls have also been recently reported.[9]

Chalazia cause localized eye symptoms such as irritation and inflammation and can induce astigmatism.[10] It is not uncommon for there to be multiple lesions, with one study reporting multiple lesions in 38% of childhood chalazia cases (most involved both eyes), with 18% of children developing at least 3 chalazia.[4]

Although chalazia are often described as the most common acquired eyelid lesions in children, few studies have reported the prevalence. In a study by Evans et al.,[4] diagnoses of all children attending a pediatric ophthalmology department (Wisconsin, USA) over 7 years were reviewed. Chalazia were present in 2% (584 of 28,433), with greater proportions in Hispanic/Latino vs non-Hispanic/Latino populations (3.8% vs 1.8%; P<0.001), American Indian or Alaska Native vs White (4.3% vs 1.7%; P <0.001), and Asian vs White (4.0% vs 1.7%; <0.001).

Rocha et al. reported chalazia as diagnosed in 34 of 932 (3.6%) pediatric cases presenting to an ophthalmology referral center in Brazil.[11] A cross-sectional observational study of 1,982,058 new patients presenting to multiple centers in India between 2010 and 2019 found an overall prevalence rate of chalazia in children of 0.95% (2656 of 280,034).[12]

Chalazia recur, although there are no prospective data on the rate or time course of chalazia recurrence. A large, multi-center study in India estimated recurrence at 9.6% (95% CI: 9.1–10.2%; the follow-up period is not reported).[12] Following surgical intervention, recurrence has been reported as associated with younger age at presentation and with a greater number of chalazion.[13]

1.2 Natural History

The natural history of chalazia has yet to be documented. However, clinical textbooks (e.g., https://www.merckmanuals.com/professional/eye-disorders/eyelid-and-lacrimal-disorders/chalazion-and-hordeolum-stye) and professional association websites (e.g., https://eyewiki.aao.org/Chalazion) frequently state that most chalazia resolve spontaneously within several days to weeks.

1.3 Current Practice

Current practice, and the evidence on which it is based, was recently evaluated in two scoping reviews.[14, 15] Applying warm compresses to the affected area is a common conservative treatment thought to help drain the blocked gland. If a chalazion persists for more than 2 months

and conservative treatments like warm compresses are ineffective, procedures such as incision and curettage, total excision, or steroid injections are commonly recommended. For chalazia that are large in size and inflamed, other treatments such as steroid ointment or anti-inflammatory eye drops are often used. Certain oral antibiotics are reported to be prescribed for their anti-inflammatory effects or when a chalazion is associated with a bacterial infection or inflammation of the surrounding eyelid tissue (cellulitis). Recent studies have shown the frequent association of chalazia with *Demodex* infestation,[1-3] leading to recommendations that *Demodex* be treated in children with chalazia.[2]

As part of the Pediatric Eye Disease Investigator Group (PEDIG) Chalazia Study planning process, a survey was designed and delivered to all PEDIG investigators (November 2022) to determine current practice patterns within the PEDIG network. Questions regarding treatment preferences were framed for each of 4 chalazia severity categories based upon inflammation and duration: inflamed <30 days, inflamed ≥30 days, non-inflamed <30 days, and non-inflamed ≥30 days. One hundred-thirty-five investigators (77 [57%] ophthalmologists, 58 [43%] optometrists) responded to the questionnaire. The most frequently used first-line treatments across all severity types (with or without other treatments) were warm compresses (76% to 94% of investigators across all 4 severity categories) and eyelid scrubs (63% to 79% of investigators across severity categories); this was followed by topical antibiotic-steroid combination eye drops (63% to 76% of investigators, across all 4 severity categories). Oral omega-3 supplementation (up to 37% of investigators) and oral antibiotics (up to 37% of investigators) were also used across all 4 severity categories. When asked whether the presence of *Demodex* would affect willingness to randomize to warm compresses/lid scrubs, the majority (79% or more) reported that it would not. Likewise, considering treatment using omega-3 supplementation, 90% or more of investigators felt the presence of *Demodex* would not influence their willingness to randomize.

1.4 Treatment Effectiveness in Pediatric Populations

Most studies of treatment effectiveness for chalazia have been conducted in adults. A recent scoping review found only sparse evidence for effectiveness of any treatment for chalazia in pediatric populations.[14] In addition, a literature search yielded only two randomized studies in children (summarized below); however, neither reported effectiveness of the standard treatment (warm compresses with or without eyelid cleansing).

In an RCT of 26 children with chalazia, Filippelli et al.,[16] compared at least 20 days of medical treatment (lid hygiene, warm compresses, and dexamethasone/ tobramycin ointment) alone versus medical treatment plus 3 months of oral probiotic supplementation. The study reported that the group treated with oral probiotic supplementation (combined with medical treatment) achieved faster chalazia resolution: 28 (21 to 39) days vs 54 (37 to 63) days; $p < 0.0001$] respectively. However, the proportion of participants achieving resolution was not reported for either group.[16]

In a pilot prospective randomized study of 26 children with chalazia (36 lesions), Mustafa et al.[17] compared different interventions. Chalazia lesions were randomized into 3 treatment groups: 1) intralesional triamcinolone acetonide 5 mg/mL injection (n=12), 2) simple incision and curettage (n=12), 3) incision, curettage, and intralesional triamcinolone acetonide injection (n=12). After 2 weeks, resolution was achieved in 9/12 (75%) lesions treated with triamcinolone injection, 9/12

(75%) with incision and curettage, and 12/12 (100%) with incision, curettage, and triamcinolone injection.

Among nonrandomized studies there also appear to be none evaluating the effectiveness of the standard treatment of warm compresses with or without eyelid scrubs in children.[14, 15, 18]

Evidence of treatment effectiveness for chalazia specifically, and ocular surface disease in general is scarce in pediatric populations. Recent Cochrane reviews on surgical and non-surgical treatment effectiveness on conditions associated with chalazia (acute internal hordeolum and blepharokeratoconjunctivitis) concluded that no randomized trials met criteria for inclusion.[19, 20] There are currently no systematic reviews summarizing treatment evidence in chalazia.

1.5 Conventional Treatment Effectiveness in Adult Populations

Because treatment evidence for chalazia and ocular surface disease in pediatric populations is so scarce, available estimates of the effectiveness of conventional treatment of chalazia were reviewed for adults.

1.5.1 Warm Compresses

Warm compresses are the most common and least invasive treatment for chalazia and have been evaluated in three previous randomized trials in adults.

In adults, reported chalazia resolution rates from treatment with warm compresses range widely (21% to 77%), with differences in study duration, in the number of times performed daily, and whether eyelid hygiene and/or antibiotic ointment was also used (see Table 1 below).[21-25]

Table 1 – Studies Reporting on Warm Compresses

Author	Study Type	Duration	N (warm compress group only)	Intervention	Outcome
Wu et al 2018[25]	RCT	4-6 weeks	N=50 adults (N=63 eyelids) Median age: 46, range 20-81 years	Hot compresses	Resolution rate 21% (95% CI: 11% to 31%)
Goawalla and Lee 2016[23]	RCT	Within 3 months	N=35 adults Aged 18+ years	Warm compresses plus chloramphenicol ointment, three times daily for 5 days	Resolution rate 46% (95% CI: 29% to 63%)
Chung et al 2007[22]	RCT	Within 4 weeks	N=12 adults Aged 38±17 years	Warm compresses, eyelid hygiene & chloramphenicol 1% eye ointment, 4 times daily	Resolution rate 58% (95% CI: 30% to 86%)
Perry and Serniuk 1980[24]	Single Arm Prospective Study	Within 12 weeks	N=37 (N=47 chalazia) Median age 32 years, range 14 months-62 years	Lid hygiene and warm saline soaks a few times a day	Resolution rate 35/47 chalazia 74% (95% CI: 71% to 95%)
Alsoudi et al 2022[21]	Retrospective	Unknown	N=959 adults Aged 38±25 years	Warm compresses with/without eyelid scrubs	Resolution rate 77% (95% CI: 74% to 79%)

1.5.2 Eyelid Cleansers

Eyelid cleansing is sometimes prescribed alongside warm compresses to clean debris from the eyelids, with the goal of further alleviating blockage of the meibomian glands and aiding treatment effectiveness. In some prior chalazia studies (adult populations)[21, 22, 24] eyelid cleansing is incorporated as part of standard treatment whereas in others, standard treatment consists of warm compresses only.

There are no prior studies comparing the effectiveness of warm compresses alone versus warm compresses with eyelid cleansing for the treatment of chalazia. However, eyelid cleansing will be added to warm compresses in the present study, to potentially enhance treatment benefit in the standard treatment arm.

Many different approaches and products are used for eyelid cleansing,[26] but few have been well studied. Some commercially-available eyelid cleansers have been evaluated in randomized trials for treatment of meibomian gland dysfunction[27] and blepharitis[28] in adults, but there are no data on the effectiveness of different eyelid cleaners in the treatment of chalazia, whether in adults or children.

OCuSOFT® Eyelid Scrub Plus Pre-Moistened Pads were selected for standard eyelid cleansing in the present study because they are commonly used in clinical practice, appear to be well-tolerated and are easy to use in a pediatric population. We are aware of one randomized study evaluating the effectiveness of OCuSOFT® Eyelid Scrub Plus: Murphy et al[29] compared OCuSOFT® eyelid Scrub Plus with Dr Organic Tea Tree Face Wash, and the BlephEx™ device, for the treatment of *Demodex* blepharitis in adults. The authors do not comment on tolerability of the treatments but all three methods were shown to reduce *Demodex* count.[29]

1.6 Oral Omega-3 Essential Fatty Acids Supplementation

Dietary supplementation with oral omega-3 essential fatty acids (EFAs) has been reported to reduce the systemic inflammatory state and improve blepharitis and meibomian gland dysfunction.[30] In other studies, dietary supplementation of oral omega-3 EFAs worked by affecting the polar lipid profiles of meibomian gland secretions in women with Sjögren's syndrome.[31] In cell-based assays, introducing omega-3 to human meibomian gland epithelial cells increased intracellular triglycerides and small neutral lipid-containing vesicles.[32] Oral omega-3 EFAs have also been proven to benefit the inflammatory disorder of rheumatoid arthritis.[33]

Although there are no prior data on the effectiveness of omega-3 treatment for chalazia, it is reasonable to anticipate a similar effect on inflammation to that seen in other ocular surface disease. Omega-3 supplementation has been used (in the form of flaxseed oil; α-linolenic acid) as a maintenance treatment in children with blepharokeratoconjunctivitis (after eyelid margin disease was controlled) with no reported side effects.[34] The American Academy of Ophthalmology also supports the use of omega-3 in pediatric eye eyelid margin disease.[35] Finally, in a PEDIG survey of chalazia treatments, about one-third (up to 37%) of investigators were using oral omega-3 supplementation as a treatment for chalazia. These factors supported the emerging rationale for studying the use of oral omega-3 treatment for pediatric chalazia.

Oral omega-3 EFA dietary supplementation in children has not been evaluated by the Food and Drug Administration (FDA). Nevertheless, the FDA has stated that omega-3 essential fatty acid supplementation (consisting of both eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) up to 5g per day is generally considered safe in adults.[36]

There are no recommended daily dose guidelines for oral omega-3 supplementation in children. In prior randomized studies of children receiving omega-3 supplementation, daily doses ranged from 100 mg (children <25kg with Attention Deficit Hyperactivity Disorder [ADHD])[37] to 4000mg (10 to 17-year-olds with high triglyceride levels).[38] In randomized trials that enrolled children as young as 4 years old, daily doses ranged from 300mg (4 to 11-year-olds with ADHD)[39] to 1300mg (3 to 8-year-olds with autism).[40]

1.7 Chalazia and *Demodex*

In recent years, evidence has emerged noting the presence of *Demodex* mites on the eyelid margins of children[1-3] and adults[41, 42] with chalazia. Of the two species of *Demodex* mite that affect human skin (*Demodex folliculorum* and *Demodex brevis*), both have been reported in association with chalazia.[1,41]

Three studies have reported the presence of *Demodex* in children with chalazia compared with a control population without chalazia. Estimates of *Demodex* prevalence in children with chalazia range from 53% to 73% compared with 0% to 36% in controls:

Xiao et al.[3] conducted a prospective case-control study in 101 consecutive children with chalazia and 42 control subjects without chalazia. All participants underwent eyelash sampling for microscopic identification and counting of *Demodex* mites. Ten eyelashes were randomly sampled from each eye and *Demodex* was found to be present in 73.3% of the chalazia group vs 35.7% of the control group ($P \leq .001$); there was also greater prevalence in those with multiple chalazia vs a single lesion (79.2% vs 58.6%, P= .035).[3] *Demodex* count was significantly higher in the chalazia group (3.06 ± 3.48) compared with the control group (0.64 ± 1.17) (≤ .001) with a greater number of mites in those with multiple chalazia vs a single lesion (3.49 ± 3.72 vs 2.00 ± 2.52; P= .043).[3]

Liang et al.[1] prospectively evaluated 4 eyelash samples from each eyelid of 47 children with chalazia and 30 age- and sex-matched controls without chalazia by microscopic identification and counting of *Demodex* mites and found a higher prevalence of *Demodex* in those with chalazia (70.2% vs 13.3%; P <.001). The greater prevalence among children with chalazia vs those without was found for both *Demodex brevis* (53.2% vs. 3.3%; P <0.001) and for *Demodex folliculorum* (55.3% vs. 10%; <0.001).

Huang et al.[2] prospectively evaluated 3 eyelash samples from 446 children undergoing surgery for chalazia and 50 children undergoing surgery for non-inflammatory eye disease (controls). The prevalence of *Demodex* was 53% in those with chalazia vs 0% in controls. In multivariate logistic regression analysis, the presence of *Demodex* was correlated with recurrent chalazia (P=0.006) and the presence of multiple chalazia (P=0.023)

While the relationship between the presence of *Demodex* infestation and the development of chalazia is not yet fully understood, several authors have suggested plausible causative mechanisms;[1-3, 43-45] these include granulomatous or giant cell reaction in response to the mites as a foreign body, mites mechanically obstructing the meibomian glands, and mites acting as vectors introducing microbes that induce an immune response.

1.7.1 Treatments to Reduce Ocular *Demodex*

Given the prevalence of ocular *Demodex* in pediatric chalazia and reasonable plausibility regarding causation, there is increasing interest in addressing the presence of *Demodex* as part of treatment for chalazia. Although there are not yet any studies evaluating the effect of *Demodex* treatment on chalazia, there is growing evidence of its effect on other eye conditions, particularly *Demodex* blepharitis.[29, 46-48]

1.7.1.1 Tea-Tree Oil

Tea tree oil (melaleuca alternifolia), or its most abundant and potent component, terpinen-4-ol,[49] are common treatments for ocular *Demodex*.[50] Terepin-4-ol has been reported to be effective in killing *Demodex* mites (in vitro), even at concentrations as low as 1%.[49] Some studies report tea tree oil concentrations of 50-100%;[47, 51] however, these higher concentrations can irritate the ocular surface and cause stinging.[47, 50, 52, 53] Commercially-available eyelid products that have been studied in clinical populations generally contain substantially lower concentrations of terpinen-4-ol (see Table 2 below). As a result, they may be better tolerated, especially in children, but might also be less effective.

Table 2 - Concentrations of Tea Tree Oil in *Demodex* Eyelid Treatments[51]

Commercially Available *Demodex* Treatment	Terpinen-4-ol (mg terpinen-4-ol per ml product)	Mite Survival Time (in Minutes)	
		Median	Range
Cliradex®	4.61	37.5	15–240
Oust™ Demodex®	0.29	90	30–150
Blephadex™	0.03	60	15–240
TheraTears® SteriLid	0.02	70	30–145
50% Tea Tree Oil	337.36	28	24–75

[1]Other eyelid products are commercially available, but there are no available data on their concentration / effectiveness.

Tea Tree Oil Treatment Effect

Evidence of tea-tree oil or terpinen-4-ol's effectiveness in eradicating *Demodex* mites is limited. In a randomized in vitro study evaluating *Demodex* mite survival time with 8 different anti-*Demodex* treatments,[51] 100% terpinen-4-ol, 100% tea-tree oil, and 100% linalool were most effective, followed by 50% tea-tree oil. Of the four commercially available eyelid cleansers studied (Cliradex®, Oust™ Demodex®, Blephadex™, TheraTears® SteriLid), only Cliradex® demonstrated in vitro anti-parasitic activity/mite survival time comparable to that of 50% tea-tree oil. The median mite survival time was 37.5 minutes for Cliradex® and 28 minutes for 50% tea tree oil (P = 0.36 by post-hoc pairwise comparative analysis). No commercially available cleansers showed comparable survival times to 100% tea tree oil.[51] It is unknown whether the relative effects found in vitro would apply to in vivo administration of the anti-Demodex solution.

While there are no prior studies evaluating the effectiveness of treatment of *Demodex* in children with chalazia, there are several clinical studies evaluating the effectiveness of Terpinen-4-ol / tea-tree oil treatments in other eye conditions (all in adult populations), including 3 randomized trials of *Demodex* blepharitis (inflammation along the edges of the eyelids caused by *Demodex*). Note that study outcomes of reduction of average Demodex mites count and/or *Demodex* mite eradication (summarized in Table 3 below) are those potentially most relevant to a study of chalazia; however, it is unknown whether these outcomes would be effective for treating chalazia. When reported, Demodex mite count/presence was determined via high magnification microscopy of epilated lashes.[29, 46-48]

260 **Table 3 - Studies of Tea Tree Oil for *Demodex* Blepharitis and Ocular *Demodex***

Author	Study Type	Population	Interventions	Duration	Outcome	Considerations/Limitations
Epstein et al[46]	RCT	N=50 adults with *Demodex* blepharitis (outcomes on n=46)	All participants received in-office microblepharoexfoliation at baseline and 1 month. Randomly assigned to Cliradex® or Sham eyelid scrubs for 1 month. At 1 month all received open label Cliradex® Scrubs twice daily for an additional month	2 months	In Cliradex® group, *Demodex* mite count was not significantly reduced at 1 month (Baseline mean: 4.7 ± 1.5; 1-month mean: 3.6 ± 1.5, P = 0.266) but was significantly different between baseline and 2 months (mean 2.6 ± 1.2; t- test: P = 0.026). In the sham treatment group, there was a statistically significant reduction in mites from baseline to 1-month (Baseline mean: 5.1 ± 1.4; 1-month mean: 3.0 ± 1.0, P = 0.015) and from baseline to 2-month mean (2.5 ± 0.9, P=0.005). Cliradex® and sham groups were not formally compared but did not appear to differ. Cliradex® was described as being mostly well tolerated; a few participants reported burning or irritation that dissipated in minutes or less.	All participants received In-office microblepharoexfoliatin treatment
Murphy et al [29]	RCT	86 adults with suspected *Demodex* folliculorum blepharitis, analyzing 69 confirmed cases; the remaining 17 assigned as controls	1) Dr Organic Tea Tree Face Wash (containing 38% terpinen-4-ol) (n=28: 22 with *Demodex*, 6 controls) 2) OCuSOFT® eyelid Scrub Plus (active ingredient 1, 2-Octanediol) (n=30: 24 with *Demodex*, 6 controls) 3) the BlephEx™ hand-held microblepharoexfoliation device (n=28: 23 with *Demodex*, 5 controls).	4 weeks	Mean (range) mite count reduced significantly with the Tea Tree Face Wash from 4.9 (0–21) at baseline to 2.2 (0–12) at 2 weeks (P=0.007) and 1.9 (0–8) at 4 weeks (p=0.001) and with BlephEx™ from 6.5 (1–25) at baseline to 3.1 (0–13) at 2 weeks (P=0.006) and 2.7 (0–9) at 4 weeks (P=0.001). Using the OCuSOFT® eyelid Scrub, changes from baseline to 2 weeks (3.8 [0–11] vs 2.5 [0–17]) were not significant (P = 0.057), but changes were significant comparing baseline with 4 weeks (3.8 [0–11] vs 1.9 [0–7]; P=0.005). The mean mite *Demodex* count was no different between the three treatment groups after 2 (P=.703) or 4 weeks (P=0.498) of treatment. Mite count was reduced in all treatment groups at 4 weeks. The authors concluded that all three methods showed good ability to reduce *Demodex* quantity.	Not a true control group
Koo et al.[47]	RCT	N=281 adults with ocular *Demodex*	1) Weekly office visits using 50% concentration tea tree oil, daily home scrubs with 10% concentration tea tree oil 2) Daily eyelid scrubs without tea tree oil (saline) (home treatment)	4 weeks	*Demodex* mite count reduction was significant in the tea tree oil group (from 4.0 ± 2.5 to 3.2 ± 2.3; P = 0.004) Reduction was non-significant in the control group (from 4.3 ± 2.7 to 4.2 ± 2.5; P = 0.27).	The outcome data were reported on only 54 (39%) of the 140 assigned to scrubs without tea tree oil vs 106 (75%) of the 141 assigned to tea-tree oil scrubs.
Huang et al[48]	Non-randomized prospective cohort	N= 80 adults with evidence of *Demodex* infestation	Oust™ *Demodex*® was used twice daily for 1 month, then once daily for 2 months (topical	3 months	Ocular *Demodex* eradication was achieved in 28 eyes (35%), and average *Demodex* mite count was significantly reduced in participants with lower baseline ocular Demodex load (ODL) (from 4.7 to 3.3; P = .043;	In the ivermectin-treated sub-group 19 (76%) of 25 achieved eradication of ocular *Demodex* whereas eradication was achieved

		plus chronic eyelid inflammation Participants were categorized as having either low ocular *Demodex* load (ODL) (1 to 7 mites per eye; n=41) or high ODL (8 or more mites per eye; n=39)	lubricants and antiallergic eyedrops also allowed) 50 participants had co-existent facial *Demodex*, 25 of whom were treated with topical ivermectin.		and participants with higher ODL (from 12.9 to 4.4, P < .001)	in only 4 (16%) of the 25 with facial *Demodex* who were not treated with topical ivermectin (P < .001).

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1.7.1.2 Lotilaner Ophthalmic Solution 0.25% (Xdemvy)

Lotilaner Ophthalmic Solution 0.25% (tradename Xdemvy) is the only FDA-approved treatment for ocular *Demodex*, with approval currently limited to treating *Demodex* blepharitis in adults (approved July 2023). Lotilaner acts by inhibiting γ-aminobutyric acid (GABA) chloride channels in *Demodex* mites, leading to paralysis and death. Lotilaner has not been FDA-approved for pediatric use or studied in pediatric populations. It has not been studied for treating chalazia in the pediatric or adult population.

FDA approval was granted based on evidence from 2 randomized clinical trials summarized in Table 4 below.

Table 4 - Studies of Lotilaner as Treatment for Ocular *Demodex*

Study	Population	Duration	Outcome
Saturn-1[54] (NCT04475432)	N=421 adults with *Demodex* blepharitis	43 days	At day 43, the lotilaner group had a greater proportion of mite eradication based on epilation and microscopic examination than the control group (67.9% vs. 17.6%; P < 0.0001). All adverse events were mild, the most common being pain on drop instillation (lotilaner 11.8% vs control 7.7%).
Saturn-2[55] (NCT04784091)	N=412 Adults with *Demodex* Blepharitis	43 days	A greater proportion of the lotilaner group compared with the control group (51.8% vs. 14.6%) (P < 0.0001) had mite eradication based on epilation and microscopic examination. 90.7% of participants using lotilaner found the drops to be neutral to very comfortable.

1.8 Rationale for Present Study

Chalazia are common eyelid lesions in children, and management requires frequent in-office participant visits and sometimes invasive treatment procedures. Nevertheless, there is a dearth of evidence for the effectiveness of any treatment for chalazia in children. In addition, estimates of treatment effectiveness for warm compresses in adults vary widely (21% to 77%).[21-25] Therefore, the true resolution rate for conservative treatment with warm compresses, with or without eyelid cleansers, is unknown, even in adults. Given the frequency with which children with chalazia present to pediatric eyecare providers and primary care physicians and the paucity of evidence for treatment effectiveness, there is a pressing need for studies.

Before conducting full-scale comparative effectiveness studies of chalazia treatments in children, it is necessary to estimate the safety and effectiveness of conservative treatment using a protocol of twice-daily warm compresses and standard eyelid cleansers to achieve improvement and resolution of chalazia. Standardized treatment is important in a randomized trial. Therefore, a Bruder® mask will be used to ensure consistent application of warm compresses, and a standard eyelid cleanser (topical OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads) will be used. The active ingredients of this OCuSOFT® eyelid cleanser are polyhexamethylene biguanide preservative (PHMB), provitamin B5 (conditioning agent), and decyl glucoside (surfactant).

Given the paucity of current evidence for treating childhood chalazia, this study is designed as a Phase II RCT with a convenience sample size. Anti-*Demodex* treatment was selected due to the high prevalence of *Demodex* in recent studies of pediatric and adult populations with chalazia,

along with a lack of evidence on its acute treatment effect. Oral omega-3 supplementation was also selected as a treatment group based on high interest among the PEDIG investigator group to explore whether oral omega-3 oral supplementation might: 1) have an acute treatment effect and/or 2) reduce the occurrence of new onset chalazia in children with a history of chalazia/chalazion. This phase II study will also establish parent and child willingness to be randomized and provide an opportunity to identify operational challenges prior to a potential full-scale RCT.

A telehealth objective for the current study is to evaluate agreement between a central reader assessment of chalazia using parental photographs, parental palpation of eyelids and a Parental Treatment Outcome Question versus a "gold standard" in-office clinical assessment. If telehealth assessments of parental photographs, parental palpation of eyelids, and the Parental Treatment Outcome Question are reliable, they could significantly affect public health by reducing outpatient visits, leading to cost savings, increased convenience, and lessened parental burden. A successful telehealth approach could also help reduce health disparities by providing timely care access to those who struggle to attend in-person office visits.

1.9 Study Objectives

The study objectives are to evaluate the safety and efficacy 1) over 8-weeks for one or more chalazia, and 2) for the prevention of new onset chalazia between 8 weeks and 12 months; in children aged 4 to <17 years treated with one of three treatment regimens:

- **OMEGA-3** Group: oral omega-3 supplementation, warm compresses, and standard eyelid cleanser for 8 weeks; followed by continued omega-3 supplementation plus standard care between 8 weeks and 12 months.
- **ANTI-*DEMODEX*** group: warm compresses and anti-*Demodex* eyelid cleanser for 8 weeks; followed by standard care, except for no omega-3 supplementation, between 8 weeks and 12 months.
- **STANDARD** group: warm compresses and standard eyelid cleanser for 8 weeks; followed by standard care, except for no omega-3 supplementation, between 8 weeks and 12 months.

Another important objective is to evaluate the agreement between an in-office clinical assessment by a masked investigator ("gold standard") versus a masked central reader assessment based on review of telehealth information (parental digital photos, parental external palpation, and Parental Treatment Outcome Question) for determining whether chalazia have improved/resolved (vs. not) at 8 weeks, across all treatment groups.

1.10 Potential Risks and Benefits of Study Treatment

1.10.1 Potential Risks of Warm Compresses

The main risk of using a Bruder® mask for application of a warm compress, as prescribed in our study, is the potential for overheating the mask, which could cause burns or damage to the skin. This risk will be minimized by providing clear written and verbal instructions to the parent and child regarding the length of time the mask should be in the microwave and how to evaluate the Bruder® mask temperature before placing it on the child's eyelids.

1.10.2 Potential Risks of Standard Eyelid Cleanser

The standard eyelid cleanser will be topical OCuSOFT® Eyelid Scrub Plus Pre-Moistened Pads. Side effects from these eyelid scrubs are rare and usually mild but can include irritation, itching, swelling, allergic reaction, or stinging of the skin or eye.[56, 57]

1.10.3 Potential Risks of Anti-*Demodex* Eyelid Cleanser

The Anti-*Demodex* eyelid cleanser that will be used is topical Cliradex® Towelettes that contain 4.61 mg terpinen-4-ol per ml product. Side effects of Cliradex® or low-concentration tea-tree oil derivatives may include irritation, burning and/or stinging of the skin or eye, contact dermatitis, itching, swelling, allergic reactions,[47, 53] and epithelial cell toxicity (in vitro).[52, 58, 59]

A study regarding the tolerability of Cliradex® reported that most adult participants graded the stinging sensation as mild and well-tolerated in the short term (1 week) of use.[58]

In another study of adults, Cliradex® was described as mostly well tolerated over one month of treatment in adults, while a few participants reported burning or irritation that dissipated in minutes or less.[46]

Higher concentrations of tea tree oil (50% or more) can irritate the ocular surface and potentially cause corneal epithelial defects.[60] These higher concentrations are typically used for in-office treatment, while lower concentrations are commercially available for home use. Corneal defects have not been reported with Cliradex®.

There are no known risks to Cliradex® and pregnancy or lactation, however there are no clinical trials evaluating the risks, or lack thereof, of Cliradex® or topical tea tree oil at these concentrations. Oral tea tree oil is considered unsafe in pregnancy and breastfeeding. According to the manufacturer, "Cliradex® is a topical product derived from a natural essential oil, tea tree oil. There have been very few studies on the use of essential oils in pregnancy and while breastfeeding, so it is recommended you consult your physician before use." Due to the lack of clear evidence, anyone who is pregnant, breastfeeding, or planning to become pregnant in the first 8 weeks of the study will be excluded.

1.10.4 Potential Risks of Oral Supplementation with Oral Omega-3 EFAs

In the context of reviewing qualified health claims that EPA and DHA may help lower blood pressure and reduce the risk of hypertension (a risk factor for coronary heart disease), the FDA has concluded that dietary supplements providing no more than 5 g/day total of EPA and DHA are safe when used as recommended in adults.[36] Children in the current study will be treated based on their weight at the time of randomization. For children aged 4 to 12 years **OR** weighing less than 40 kg [88 lbs], a single 5 mL dose will be used (750 mg EPA 250 mg DHA), providing 1 g of omega-3 supplement per day. For children ≥13 years **AND** weighing 40 kg [88 lbs] or more, a single 10 mL dose will be used (1500 mg EPA and 500 mg DHA), providing 2 g of omega-3 supplement per day.

Side effects of oral omega-3 supplementation are usually mild and include unpleasant taste, bad breath, heartburn, nausea, gastrointestinal discomfort, diarrhea, headache, and odoriferous sweat.[61-63]

In a study using higher doses of omega-3 (4000mg) in 10 to 17-year-olds with high triglyceride levels,[38] 6 of 42 subjects experienced an adverse event related to the study medication (gastrointestinal symptoms, fishy taste, and frequent nosebleeds). These events were rated mild to moderate in nature and resolved spontaneously (omega-3 dose reduced in 2 of 6 cases).

Oral omega-3 dietary supplements, such as fish oil, might prolong blood clotting times when taken with warfarin (Coumadin).[63] As a precaution, children taking warfarin and other medications affecting blood coagulation will be excluded from the study. Children with a history of clotting disorders will also be excluded.

1.10.4.1 Oral Supplementation with Omega-3 Essential Fatty Acids and Pregnancy

Oral supplementation with omega-3 EFAs is often recommended during pregnancy. An RCT evaluating DHA 1000 mg per day vs. 200 mg per day during pregnancy found that those who received the higher dose had fewer preterm births and fewer serious adverse events.[64] Omega-3 supplementation is therefore not contraindicated for pregnant females and is recommended as a supplement in prenatal vitamins, therefore any participant anticipating a pregnancy during the duration of the study will not be enrolled due to confounder of needing to take Omega-3 supplements.

1.11 Risk Assessment

The expected adverse events from oral supplementation with omega-3 essential fatty acids, warm compresses with a Bruder® mask, and eyelid cleanser with OCuSOFT® Eyelid Scrub Plus Pre-Moistened Pads and Cliradex® Towelettes are summarized in section 1.10. These possible adverse events are non-significant and pose a minimal risk to participants.

The investigators believe the protocol's level of risk falls under DHHS 46.404, which is research that does not involve greater than minimal risk. Adequate provisions will be made for soliciting the assent of the children and permission of their parents or legally authorized representative as instructed by the IRB.

The use of omega-3 supplementation, Cliradex® Towelettes, and OCuSOFT Eyelid Scrub Plus Pre-Moistened Pads, will require an IND submission to the FDA.

1.12 General Considerations

The study is being conducted in compliance with the policies described in the PEDIG network policies document, the ethical principles in the Declaration of Helsinki, the protocol described herein, and the standards of Good Clinical Practice (GCP). When feasible, data will be directly collected in electronic case report forms and considered the source data.

Chapter 2: Study Enrollment and Screening

2.1 Participant Recruitment and Enrollment

The study plans to enroll up to 250 participants for whom informed consent and child assent (if required) will be obtained, with the goal of randomizing at least 168 participants.

Study participants will be recruited from PEDIG clinical centers in the United States. All eligible participants will be included regardless of sex, race, or ethnicity. There is no restriction on the number of participants to be enrolled by each site.

As the enrollment goal approaches 168 randomized participants, sites will be notified of the end date for recruitment. Participants whose parents have signed an informed consent form can be enrolled until the end date, which means the expected recruitment of 168 randomized might be exceeded. A maximum of 200 participants may be randomized.

2.1.1 Informed Consent and Authorization Procedures

A child is considered for the study after undergoing a routine eye examination (by a study investigator as part of the standard of care) or from a referral by another medical provider who identifies a chalazion (or chalazia) appearing to meet the eligibility criteria. The study will be discussed with the child's parent(s) or legally authorized representative (LAR) - referred to subsequently as parent(s). Parent(s) who express an interest in the study will be given a copy of the informed consent form to read. Written informed consent must be obtained from all parents and written assent from children age 13 years or older on the main informed consent form, and written child assent must be obtained from children age 7 years or older on the stand-alone assent form, respectively, before performing any study-specific procedures that are not part of the child's routine care.

If the participant turns 7 years of age after initial consent, then their assent must be obtained prior to performing any additional study-specific procedures, including data collection. If assent cannot be obtained, then study procedures, including data collection, cannot continue.
If the participant and/or parent(s) are not fluent in written and spoken English, then the consent and/or assent forms, as well as all other participant/parent-facing materials (e.g., dosing instructions, questionnaires), used must be those that have been translated into the language of fluency for the participant/parent(s). Further, a qualified interpreter must be available for the consent process and for all subsequent study-related interactions.

A child is considered enrolled when the informed consent form and assent form (if applicable) have been signed.

2.2 Eligibility Criteria

2.2.1 Participant Inclusion Criteria

Individuals must meet all the following inclusion criteria by clinical exam, chart review, and discussion with the family to be eligible:

1. Age 4 to <17.0 years

2. At least one chalazion measuring >3 mm, visible without special instrumentation.
3. Chalazion onset (all current lesions) within the past 6 months by parental report.
4. Willing to forgo non-study use of omega-3 supplements (i.e., pills, liquid, gummies) for the duration of the study.
5. Prior or current treatment with warm compresses, eyelid cleanser/scrubs, topical antibiotic, and/or topical steroids IS allowed.
    a. Topical antibiotics and topical steroids must be discontinued at study enrollment
    b. All non-study treatments for chalazia must be discontinued at enrollment for the first 8 weeks of the study.
6. Parent seeking treatment for child's chalazion/chalazia.
7. There is a working microwave in the home.
8. Parent possesses and brings with them to the enrollment visit a smartphone or other digital device that can take and digitally transmit photographs.
9. Parent is able and willing to take photographs and upload them to the PEDIG website.
10. Parent is able and willing to receive text or email messages during study.
11. The parent and participant are willing to comply with the assigned treatment regimen after an in-office trial of Cliradex® and oral omega-3 supplementation.
12. Willing to consume fish-based oral supplements.
13. Contact lens correction of refractive error is allowed.

2.2.2 Participant Exclusion Criteria

Individuals meeting any of the following exclusion criteria by clinical exam, chart review and discussion with the family, will be excluded from study participation:

1. Current chalazion/chalazia on any eyelid present for over 6 months by parental report
2. Use of oral omega-3 supplements (i.e., pills, liquid, gummies) in the last 2 months
3. Use of oral antibiotics or oral steroids within the prior 2 weeks
4. Use of anti-*Demodex* treatment in the last 2 months
5. Current or prior use of implanted steroids
6. Prior surgery or injection for chalazia on any eyelid within the previous 6 months
7. Surgery or injection planned for chalazia in next 8 weeks.
8. Presence of ptosis as defined by 2-mm or smaller marginal light-reflex distance 1 (MRD-1) (distance from corneal light reflex and bottom of upper eyelid). This includes ptosis covering the visual axis. Sectoral mechanical ptosis in the area of chalazia is allowed if the visual axis is not obscured, and the investigator does not deem the ptosis visually significant.
9. History of ptosis surgery.
10. Any conjunctivitis (e.g., phlyctenular keratoconjunctivitis, allergic, bacterial, and/or vernal) requiring treatment *other than* antihistamines, artificial tears, and/or cold compresses.
11. Intraocular inflammation of any kind within the last month.
12. Intraocular conditions that in the investigator's opinion may require use of oral steroids, or systemic immunotherapy within the next year.
13. Current corneal erosion, infiltrates, ulcers, significant neovascularization (within 3 mm of optical center), or history of corneal graft or central corneal scarring.
14. Known hypersensitivity or allergy to Cliradex® Towelettes ingredients including terpinen-4-ol, a tea tree oil derivative.

15. Known hypersensitivity or allergy to OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads or its ingredients (Polyhexamethylene biguanide preservative (PHMB), provitamin B5, and decyl glucoside).
16. Known hypersensitivity or allergy to Bruder® mask.
17. Ocular rosacea.
18. Known hypersensitivity or allergy of fish or seafood.
19. Known hypersensitivity or allergy to oral omega-3 supplements.
20. Unable to consume liquid supplements orally.
21. Consumption of fish 4 or more times a week (on average).
22. Bleeding disorder or taking any anticoagulants/antiplatelet medications.
23. Daily use of aspirin, non-steroidal anti-inflammatory (NSAID), or blood-pressure-lowering medications (see 1.6.4).
24. Current chemotherapy treatment.
25. Current use of orlistat (Xenical or Alli).
26. Current use of any other medication that the investigator feels may significantly interact with omega-3 supplementation other than oral contraceptives.
27. Diagnosis of any of the following conditions:
    - diabetes mellitus, heart rhythm disorder, underactive thyroid, liver disease, or pancreatic disease
28. Moving outside the area in the next 12 months.
29. Other family member participating in the study.
30. Immediate family member (i.e., parent or legally authorized representative, sibling) is an investigator, coordinator, or certified tester directly affiliated with this study or an employee of the JAEB Center for Health Research.
31. Has previously been randomized as a participant in this study.
32. Developmental delays/sensory sensitivities that parents or the investigator feel would interfere with study treatment.
33. Pregnant, lactating or planning to become pregnant during study participation.

2.3 Procedures at Enrollment

After informed consent is obtained (and written or verbal assent, if applicable), a potential participant will be evaluated for study eligibility by reviewing their medical history and conducting clinical testing as follows.

2.3.1 Demographic and Historical Information

The following demographic and historical information will be collected: date of birth, weight, sex, race, ethnicity, medical history, concomitant medications, whether the participant is pregnant, planning to become pregnant, or lactating, and ocular history. The ocular history will detail the number of chalazia in the past year, the estimated duration of the current chalazion, and current and prior treatments for chalazia (if applicable).

2.3.2 Clinical Procedures at Enrollment

All examination procedures must be performed on the same day as enrollment. Testing may be performed after cycloplegia if necessary. Refer to Chapter 5 herein and the CH01 Site Manual of Procedures for a complete description of testing procedures.

Procedures must be performed in the specified order.

1. In-office Trial of Omega-3 Supplement
   - A trial 5 mL dose of oral omega-3 supplementation will be consumed in the office to assess tolerability.
2. Clinical Examination by Investigator
   - An assessment of chalazia by slit lamp examination of the anterior segment of the eye, including palpation of the eyelids, will be performed.
   - The presence of collarettes will be assessed during the slit lamp exam. If collarettes are present, the upper eyelid with the most collarettes will be graded on a 5-point scale.
3. In-office Trial of Cliradex® Towelettes
   - A trial using one Cliradex® towelette on the upper and lower eyelids of the non-affected eye if unilateral chalazia or right eye if bilateral chalazia will be performed in the office to assess tolerability and demonstrate proper use for home application.
   - A trial 5 mL dose of oral omega-3 supplementation will be consumed in the office to assess tolerability.
4. Body Weight Measurement
   - The participant's body weight will be measured (in the office) to the nearest pound.
5. Parental Photographs
   - As instructed by the investigator or coordinator, the parent will take photographs that best show the lesion(s); these will be used for reference at the 8-week outcome visit.
   - Three mandated photographs and optional, additional photographs, using the parent's smartphone or other digital device, will be taken showing both the upper and lower eyelids of both eyes:
     - Photograph 1: both eyelids closed
     - Photograph 2: both eyelids open, and eyes looking straight ahead
     - Photograph 3: both eyelids open and eyes looking up
     - Photograph 4+ (optional): additional photograph(s) that show the lesion(s) the best
   - Photos will be uploaded to the PEDIG website by the parents as instructed by the investigator or coordinator.

2.4 Confirmation of Eligibility for Randomization

Randomization will occur at the end of the enrollment visit after confirming the participant meets all eligibility criteria. Participants who do not meet the eligibility criteria for randomization will be withdrawn from the study. However, participants who do not meet eligibility criteria based on having chalazia treatments too recently (e.g., omega-3 supplementation, oral steroids, oral antibiotics) may be re-considered for randomization if they meet all eligibility criteria at a later date.

Prior to randomization, the study requirements should be discussed again with the parent and child so there is reasonable assurance that the participant will adhere to the study requirements.

2.5 Randomization

Eligible participants will be randomly assigned 1:1:1 to the following groups (treatments are described further in Chapter 3):

1. **OMEGA-3 Group**: oral omega-3 supplementation, warm compresses, and standard

eyelid cleanser for 8 weeks; followed by continued omega-3 supplementation plus standard care between 8 weeks and 12 months.

2. **ANTI-*DEMODEX* Group**: warm compresses and anti-*Demodex* eyelid cleanser for 8 weeks; followed by standard care, except for no omega-3 supplementation, between 8 weeks and 12 months.
3. **STANDARD Group**: warm compresses and standard eyelid cleanser for 8 weeks; followed by standard care, except for no omega-3 supplementation, between 8 weeks and 12 months.

A Master Randomization List using a permuted block design stratified by the following two age/weight groups specifying the order of treatment group assignments will be used for randomization:

- Children 4 to 12 years old at randomization **OR** weighing less than 40 kg (88 lbs) if age 13 or older.
- Children 13 years or older at randomization **AND** weighing 40 kg (88 lbs) or more.

Once the website randomization process is complete, a study participant is randomized. The participant will be included in that group for analysis regardless of whether the assigned treatment is received. Thus, the investigator must not randomize the participant unless convinced that the child is eligible, and the family will accept whichever treatment group is assigned through randomization.

Chapter 3: Treatment in Randomized Trial

3.1 Treatment

Treatment should start on the day after enrollment. Participants will be treated depending upon random treatment assignment (see 3.1.1, 3.1.2 or 3.1.3) and 3.1.4. Investigators must not start any additional treatment for chalazia (other than the randomized study treatment outlined below) before the 8-week visit.

3.1.1 OMEGA-3 Group

- Oral omega-3 supplementation, followed by continued omega-3 supplementation plus standard care between 8 weeks and 12 months.
- Twice daily warm compresses with a Bruder® mask (10 minutes in the morning and the evening) and twice daily eyelid cleansing with OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads for 8 weeks.
- All other treatments between 8 weeks and 12 months (e.g., warm compresses, eyelid cleanser/scrubs, topical steroids, antibiotics, surgery) are at investigator discretion.

3.1.1.1 Oral Omega-3 Supplementation

If prescribed, the Physician Recommended Nutraceuticals (PRN) product Eye Omega Benefits for Kids will be used to provide liquid oral omega-3 supplementation in this study. Eye Omega Benefits for Kids is derived from fish-oil, is gluten-free, dairy-free and has a fruity flavor. A marked dosing cup and/or syringe will be provided to administer the liquid omega-3 product.

For participants 4 to 12 years old **OR** weighing less than 40 kg (88 lbs), a single daily 5 mL dose will be used (750 mg eicosapentaenoic [EPA] 250 mg docosahexaenoic acid [DHA]), providing 1 g of oral omega-3 supplementation.

For participants ≥13 years old **AND** weighing 40 kg (88 lbs) or more, a single daily 10 mL dose will be used (1500 mg eicosapentaenoic [EPA] and 500 mg docosahexaenoic acid [DHA]), providing 2 g of oral omega-3 supplementation.

Participants will be instructed to take the Omega-3 supplement by mouth once daily, at approximately the same time of day for 12 months. Parents should ensure their child takes the Omega-3 supplement every day. A calendar will be provided for the parent to record adherence to the Omega-3 supplement (if prescribed).

6-month Omega-3 Supplementation Dosage Adjustment

All participants will be weighed at the 6-month visit. Dosage may be modified at 6 months based on weight and age, based on above criteria.

3.1.2 ANTI-*DEMODEX* Group

- No oral omega-3 supplementation
- Twice daily warm compresses with a Bruder® mask (10 minutes in the morning and the evening) followed by twice daily eyelid cleansing with Cliradex® Towelettes per treatment protocol for 8 weeks.
- Followed by standard care, except for no omega-3 supplementation, between 8 weeks and

12 months.
- All other treatments between 8 weeks and 12 months (e.g., warm compresses, eyelid cleanser/scrubs, topical steroids, antibiotics, surgery) are at investigator discretion.

3.1.2.1 Anti-*Demodex* Eyelid Cleanser

Cliradex® Towelettes are eyelid wipes formulated with a non-irritating skin cleanser containing a proprietary formulation of steam-distilled tea tree oil (4.61 mg/mL terpinen-4-ol) that are designed to eradicate *Demodex* mites. The towelettes are pre-moistened and individually wrapped for single use. Detailed written instructions will be provided to the participant and parent.

3.1.3 STANDARD Group

- No oral omega-3 supplementation.
- Twice daily (10 minutes in the morning and the evening) warm compresses with a Bruder® mask followed by twice daily eyelid cleansing with OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads per treatment protocol for 8 weeks.
- Followed by standard care, except for no omega-3 supplementation, between 8 weeks and 12 months.
- All other treatments between 8 weeks and 12 months (e.g., warm compresses, eyelid cleanser/scrubs, topical steroids, antibiotics, surgery) are at investigator discretion.

3.1.3.1 Standard Eyelid Cleanser

OCuSOFT® eyelid Scrub Plus Pre-Moistened Pads are individually wrapped pre-moistened pads containing a mild, non-irritating eyelid cleanser. Daily eyelid hygiene is recommended to remove contaminants from the eyelids and eyelashes. Detailed written instructions for use will be provided.

3.1.4 Warm Compresses

All participants will be prescribed twice daily warm compresses with a Bruder® mask (10 minutes in the morning and the evening) followed by the appropriate eyelid cleanser (based on randomization assignment) per treatment protocol 7 days per week for 8 weeks.

The parent or the participant (with parental supervision) should administer the warm compresses and eyelid cleanser treatment.

A calendar will be provided to record adherence with warm compresses and eyelid cleanser.

3.1.4.1 Bruder Mask

The Bruder® Moist Heat Eye Compress (elsewhere referred to as Bruder® mask) uses patented MediBeads® Technology to generate moist heat in two fabric pods. The Bruder® mask is heated using a microwave oven for 20 seconds and worn so one pod covers each eye for about 10 minutes. If additional heat is needed, the Bruder® mask may be heated for additional 5 second increments not to exceed 30 seconds.

Moisture is absorbed into the center of the beads, resulting in even, extended, moist heat. Detailed written instructions for use will be provided.

Chapter 4: Randomized Trial Follow Up

4.1 Study Visits and Contacts After Randomization

Study visits, phone contacts, and assessments are timed from randomization.

Study Visit / Contact / Assessment	Target Day (Post-Randomization)	Target Window	Allowable Window
2-Week Phone Call	Day 14	Day 14 to 21	Day 10 to 34
8-week Parental Photography & Parental Treatment Outcome Question[1]	Day 54	±2 weeks Day 40 to 70	6 to 12 weeks Day 40 to 84
8-week Office Exam - Primary Outcome	Day 56	±2 weeks Day 42 to 70	6 to 12 weeks Day 42 to 84
6-month exam Secondary Outcome	Day 183	±2 weeks Day 169 to 189	±1.5 months Day 138 to 228
12-month exam Secondary Outcome	Day 365	±2 weeks Day 351 to 370	±1.5 months Day 320 to 410
Text/Email Messages[2]	Days 30, 54, 90, 120, 150, 210, 240, 270, 300, and 330	+0 to 2 weeks	+0 to 2 weeks

[1]Target day, window, and allowable window assume that it is performed within 2 days prior to the 8-week office exam. It can be done on the day of the 8-week office exam, provided the photos are taken prior to the in-office exam.

[2] Text/email messages will be sent at 1 month to encourage adherence with protocol treatment, at 8 weeks to remind the parent to complete home tasks within 2 days before the 8-week visit (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question), and at months 3, 4, 5, 7, 8 9, 10 and 11 for parent to complete Parental New Chalazia Survey.

Additional visits may be scheduled at investigator discretion. Adverse event data may be reported and collected at any time during the study.

4.2 Side Effects of Treatment

Reporting of adverse events is described in Chapter 6. Prior to deviating from the treatment protocol or prescribing non-protocol treatment, the situation should be discussed with and approved by a Protocol Co-chair. For sites limited to one investigator and one coordinator available, the investigator will remain masked until the 8-week primary outcome visit. If needed, any adverse events reported prior to the 8-week outcome can be reviewed by the Protocol Co-chair.

4.3 2-Week Phone Call

Participants' parents will receive a phone call from site personnel 14 days after enrollment. The purpose of the call is to encourage treatment adherence with their randomized treatment and to collect data on adverse events.

If not already scheduled, the 8-week office visit will be scheduled at this time. The parent will be reminded to take and upload the photographs, perform palpation of eyelids, and complete the Parental Treatment Outcome Question (i.e., for at-home assessments of chalazia resolution) to the PEDIG website within 2 days prior to the 8-week office visit (see section 4.4). In addition, a text/email message will be sent to the parents prior to the 8-week office visit reminding them to complete the home assessment, with instructions and a link for uploading photos.

Participants will be reminded to continue using warm compresses and eyelid cleanser twice daily and to continue the oral study medication as prescribed once a day (if randomized the Omega-3 group) until the 8-week office examination.

4.4 8-Week Parental Home Assessments

Parents will have been provided (at enrollment) with written instructions regarding the 8-week home assessments (photographs, palpation of eyelids, Parental Treatment Outcome Question, photographs) and reminded on the 2-week phone call and by text or email to complete them at home within two days before the 8-week primary outcome exam, or earlier on the same day as the 8-week primary outcome exam if they have not been completed at home.

Specifically, the parent will complete the following:

1. Taking (and saving) photographs of their child's eyelids on their smartphone or other digital device as was done at enrollment (see section 2.3.2), using the same device if possible.
2. Reviewing the photographs taken at enrollment (saved on their smartphone or other digital device) and comparing them with the photographs taken at 8 weeks.
3. Palpating the child's eyelids. Written instructions on how to palpate eyelids will be provided to the family, as well as a link to an instructional video.
4. The parent will answer the following Parental Treatment Outcome Question:

   Q. Since enrolled on [autopopulated date of enrollment exam], are the bump(s) on your child's eyelids: Worse / Unchanged / Improved / Resolved?

   The following descriptions will be provided to the parent for each of the outcome categories:

   - **Worse:**
     - Any of the original bump(s) on any eyelid are larger OR
     - There are new bumps on any eyelid
   - **Unchanged:** there is very little change in the original bump(s) on any eyelid, and there are no new bumps on any eyelid
   - **Improved:** all original bump(s) on every eyelid are at least 50% smaller (i.e., half the size) and there are no new bumps on any eyelid
   - **Resolved:** there are no bumps on any eyelid and the child's eyelids are back to normal

4.5 Primary Outcome Office Visit at 8-Weeks

All examination procedures must be performed on the same day. Testing may be performed after cycloplegia if necessary. Refer to Chapter 5 herein and the CH01 Site Manual of Procedures for a complete description of testing procedures.

Testing order is not mandated, except that procedures #3-5 must be performed before procedures #6-7. Items 1-2 must be performed by study personnel who are unmasked to treatment assignment:

1. Medical History
   Including questions about the following:
   a. Treatment history and new onset chalazia not present at enrollment (approximate onset and location will be recorded).
   b. Occurrence of adverse events
   c. Concomitant medications
   d. Whether the participant is pregnant, lactating or planning to become pregnant during study participation
      i. If yes, the participant is permitted (but not required) to use omega-3 supplementation during the remainder of the study regardless of randomized treatment assignment.
2. Treatment Adherence Assessment - Home calendar logs (if brought to the visit) will be reviewed (section 4.8). Assessments of adherence with warm compresses and eyelid cleanser and with study medication will be recorded on the Follow-up Examination Form.

Items #3-5 will be performed by an investigator (masked examiner) who has not discussed the medical history and treatment adherence with the participant/parents, and who performed the assessments at enrollment if possible:

3. Clinical Examination by Investigator
   - An assessment of chalazia by slit lamp examination of the anterior segment of the eye, including palpation of the eyelids, will be performed.
   - The presence of collarettes will be assessed during the slit lamp exam. If collarettes are present, the upper eyelid with the most collarettes will be graded on a 5-point scale.
4. Investigator Treatment Outcome Question (section 4.5.1) Based on Clinical Exam Only ("gold standard") – investigator assessment of outcome based on:
   i. Complete 8-week office exam (i.e., slit lamp exam and palpation)
   ii. Enrollment in-office exam
   iii. Parental photographs taken at enrollment
5. Investigator Treatment Outcome Question (section 4.5.1) Based on Clinical Exam AND Telehealth Data – investigator will repeat their assessment of outcome after review of:
   i. 8-week Parental Assessment (parental photographs, parent palpation of eyelids, and Parent Treatment Outcome Question).

Following the masked exam, the following will be completed:

6. Unmask Treatment Group
   a. The coordinator will reveal the participant's treatment group so that the investigator who served as masked examiner may be better informed to prescribe the investigator-discretion component of treatment.

7. Prescribe Treatment
    a. Treatment between 8 weeks and 12 months is at investigator discretion except for 1) continuation of omega-3 supplementation in the **OMEGA-3** group, and 2) no omega-3 supplementation in the **ANTI-DEMODEX** and **STANDARD** treatment groups

The **OMEGA-3** group will complete calendar logs throughout the study; however, anti-*Demodex* and standard eyelid scrub groups will not receive protocolized treatment after the 8-week visit and will not be asked to continue treatment logs past the 8-week primary assessment.

4.5.1 Investigator Treatment Outcome Question

As part of the 8-week visit, the investigator will complete the following Treatment Outcome Question twice. First based on clinical exam only ("gold standard" as defined in section 4.5, assessment 4; and second based on clinical exam and the telehealth (as defined in section 4.5, assessment 5).

Q. Since enrolled, is the overall appearance of the chalazia, including all eyelids: Worse / Unchanged / Improved / Resolved?

Any of the original bump(s) on any eyelid a

- **Worse:**
    - re larger OR
    - There are new bumps on any eyelid
- **Unchanged:** there is very little change in the original bump(s) on any eyelid, and there are no new bumps on any eyelid
- **Improved:** all original bump(s) on every eyelid are at least 50% smaller (i.e., half the size) and there are no new bumps on any eyelid
- **Resolved:** there are no bumps on any eyelid and the child's eyelids are back to normal

Note that the same question will be used for the masked central reader assessment of the 8-week parental telehealth data.

4.6 Text / Email Messages

Text/email messages will be sent from the coordinating center to the participant's parent at 1 month, 8 weeks and at 3, 4, 5, 7, 8, 9, 10, and 11 months. The 1-month text/email message at will promote adherence with study treatment. The 8-week text/email message will remind the parent to complete home tasks within 2 days before the 8-week visit (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question). Beginning at 3-months, each text/email message will ask all parents to complete a brief survey regarding new-onset chalazia (i.e., not previously reported at a visit or on a previous Parental New Chalazia Survey) and to upload photographs to the PEDIG website if new chalazia are present.

4.7 Office Visits at 6 and 12-Months

All examination procedures must be performed on the same day. Testing may be performed after cycloplegia if necessary. Refer to Chapter 5 herein and the CH01 Site Manual of Procedures for a complete description of testing procedures.

Testing order is not mandated. All testing is unmasked.

1. Medical History - Including questions about the following:
    a. Chalazia treatment used since the last visit including surgery/injections
    b. Presence of new chalazia (now resolved) that were not self-reported on the PEDIG website (approximate onset and location will be recorded)
    c. Occurrence of adverse events
    d. Concomitant medications.
    e. Whether the participant is pregnant, lactating or planning to become pregnant during study participation (6-months only)
        i. If yes, the participant is permitted (but not required) to use omega-3 supplementation during the remainder of the study regardless of randomized treatment assignment.
2. Treatment Adherence Assessment (**OMEGA-3** group only) - Home calendar logs (if brought to the visit) will be reviewed (section 4.9) in the Omega-3 group only. Assessment of adherence with Omega-3 intake will be recorded on the Follow-up Examination Form.
3. Body weight measurement - participant weight will be recorded to the nearest pound (6-months only).
4. Clinical Examination by Investigator
    - An assessment of chalazia by slit lamp examination of the anterior segment of the eye, including palpation of the eyelids, will be performed.
    - The presence of collarettes will be assessed during the slit lamp exam. If collarettes are present, the upper eyelid with the most collarettes will be graded on a 5-point scale.
5. Investigator will review parent-completed new-onset chalazia logs and record presence and location of all chalazia reported by the parent since the last exam
6. Prescribe Treatment – see section 4.9

4.8 Adherence to Study Treatment

The **OMEGA-3** group will complete calendar logs throughout the study; however, the **ANTI-*DEMODEX*** and **STANDARD** groups will not receive protocolized treatment after the 8-week visit and will not be asked to continue logs past the 8-week primary outcome visit.

Adherence to omega-3 supplementation (if randomized to omega-3) will be assessed by reviewing the home calendar logs at the 8-week, 6-month and 12-month outcome exams. The proportion of calendar days that study medication was used will be categorized as either poor (<25%), fair (25% to <50%), good (50% to <75%), or excellent (>=75%).

Adherence to warm compresses and the prescribed eyelid cleanser will also be assessed by reviewing the home calendars at the 8-week outcome visit. The proportion of calendar days that warm compresses and the prescribed eyelid cleanser were used will be categorized as either poor (<25%), fair (25% to <50%), good (50% to <75%), or excellent (>=75%).

4.9 Non-Randomized Treatment for Chalazia

No treatments for chalazia other than Omega-3 supplements (if prescribed), warm compresses, and randomized eyelid cleanser as per study protocol are permitted during the first 8 weeks. If in the investigator's opinion the participant develops infection, inflammation, or other conditions that warrant outside study treatment before 8 weeks, the investigator must call the protocol chair to discuss the case and obtain approval for an exception before initiating non-randomized treatment. If non-randomized treatment is initiated prior to 8 weeks, an early 8-week primary outcome exam will be completed to assess the chalazion/chalazia prior to starting the other treatment.

Between 8 weeks and 12 months, all treatments for chalazia (e.g., warm compresses, eyelid cleanser/scrubs, topical steroids, antibiotics, surgery) are at investigator discretion, with the following exceptions:

- The **OMEGA-3** group must continue daily omega-3 supplementation.
- The **ANTI-*DEMODEX*** and **STANDARD** groups must not receive any omega-3 supplementation.

4.10 Central Reader Assessment of Telehealth Data (8-week Parental Assessment)

A masked central reader will asynchronously review the 8-week parent photos and assess the quality as excellent, good, or poor.

Regardless of photo quality, the masked central reader will answer the Investigator Treatment Outcome Question (section 4.5.1) based on review of the following data:

1. The 8-week Parental Assessment (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question)
2. The enrollment clinical exam
3. The parental photographs taken at enrollment

The masked central reader cannot evaluate the telehealth data for participants at their own clinical site.

Chapter 5: Testing Procedures

5.1 Clinical Assessments

The following study procedures will be performed as specified in the CH01 Manual of Procedures and in the protocol above. All reasonable efforts will be made to maintain masking for all study personnel if necessary.

5.1.1 Clinical Examination

An assessment of chalazia by slit lamp examination of the anterior segment of the eye, including palpation of the eyelids, will be performed per the investigator's usual clinical practice, and the following will be documented: number of chalazia on each eyelid, noting the location, size, and appearance of the largest chalazion on each lid; plugging of Meibomian glands; grading of Meibomian gland expression; eyelid margin inflammation; corneal involvement, whether *Demodex* lid debris are visible; and grading of collarettes (standard photographs in MOP) if present (see section 2.3.2 and 4.5). The clinical examination takes ~5 minutes to complete.

5.1.2 Forms / Questionnaires

Parental Treatment Outcome Question:

Completed by parent after home assessments within two days prior or on the day of the 8-week primary outcome exam (see section 4.4). This will take ~ 3 minutes to complete.

Parental New Chalazia Survey

A brief survey is completed at 1, 3, 4, 5, 7, 8, 9, 10, and 11 months (approx. 5-10 minutes). This includes uploading photographs if new chalazia have occurred.

5.1.3 In-office Trial of Cliradex® and Omega-3 Supplement

A trial of using one Cliradex® towelette to cleanse the upper and lower eyelids of the non-affected eye if unilateral chalazia or right eye if bilateral chalazia will be performed in the office at enrollment to determine tolerability and demonstrate home use. A trial 5 mL dose (for all participants) of oral omega-3 supplementation will be consumed in the office to assess tolerability. The in-office trial takes ~4 minutes to complete.

5.1.4 Parent Photographs

Parents will be instructed to take at least 3 photographs using a smartphone or other digital device 1) at the enrollment visit, 2) within 2 days prior to or on the day of the 8-week office visit, and 3) anytime a chalazia develops between 8 weeks and 12 months. These photographs will then be uploaded to the PEDIG website. Taking and uploading the photographs takes ~10 minutes to complete.

Chapter 6: Unanticipated Problems and Adverse Event Reporting

6.1 Unanticipated Problems

Site investigators will promptly report to the JCHR on an eCRF all unanticipated problems meeting the criteria below. Sites must report Unanticipated Problems to the IRB within seven (7) calendar days of recognition. For this protocol, an unanticipated problem is an incident, experience, or outcome that meets all the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied

- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)

- Suggests that the research places participants or others at a greater risk of harm than was previously known or recognized **(**including physical, psychological, economic, or social harm)

The JCHR also will report to the JCHR IRB all unanticipated problems not directly involving a specific site, such as unanticipated problems that occur at the JCHR or another participating entity, such as a pharmacy or laboratory. These instances must be reported to the JCHR IRB within seven (7) calendar days of recognition. The Director of the Human Research Protection Program will report to the appropriate regulatory authorities if the JCHR IRB determines that the event indeed meets the criteria of an Unanticipated Problem that requires further reporting.

6.2 Adverse Events

6.2.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a study participant, irrespective of the relationship between the adverse event and the drug(s) under investigation.

To further clarify, an adverse event is any unintended disease or injury, or untoward clinically significant symptom or clinical sign (including abnormal laboratory findings) in a research participant that manifests while in the study if it was not present before enrolling in the study, or if it was present before enrolling, it has increased in severity, frequency or type since enrolling in the study. For this purpose, a participant is considered enrolled once the participant has signed the consent form. For clarity, abnormalities identified as part of study screening (e.g., laboratory abnormality, physical exam abnormality) are not considered AEs even though they may have been identified after consent was signed.

Serious Adverse Event (SAE): Any untoward medical occurrence that:

- Results in death.
- Is life-threatening (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions (e.g., sight threatening).
- Is a congenital anomaly or birth defect in the offspring of a participant.
- Is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the participant or may require medical/surgical intervention to prevent one of the outcomes listed above).

6.2.1.1 Reportable Adverse Events

All reportable Adverse Events, whether volunteered by the participant's parents, discovered by study personnel during questioning, or detected through physical examination, laboratory testing, or other means, will be reported online on an adverse event form. The Medical Monitor will review each adverse event form to verify the coding.

6.3 Relationship of Adverse Event to Study Drug or Procedure

The study investigator will assess the relationship of each adverse event to be *related* or *unrelated* to the study treatment or procedure by deciding if there is a reasonable possibility that the adverse event may have been caused by the treatment or study procedure. The Medical Monitor also will make this assessment, which may or may not agree with that of the site investigator. Reporting requirements will be based on the Medical Monitor's assessment. The investigator brochure that will be developed will list potential adverse events that may be expected.

To ensure consistency of adverse event causality assessments, investigators should apply the following general guideline when determining whether an adverse event is related:

Yes
There is a plausible temporal relationship between the onset of the adverse event and administration of the study treatment, and the adverse event cannot be readily explained by the participant's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study treatment.

No
Evidence exists that the adverse event has an etiology other than the study treatment (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication), and/or the adverse event has no plausible temporal relationship to study treatment administration.

6.4 Severity (Intensity) of Adverse Event

The severity (intensity) of an adverse event will be rated by the site investigator and Medical Monitor. A severity assessment is a clinical determination of the maximum intensity of the

adverse event. Thus, a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

- MILD: Usually transient, requires no special treatment, and does not interfere with the participant's daily activities.
- MODERATE: Usually causes a low level of inconvenience, discomfort, or concern to the participant and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures, and the participant is able to continue in the study.
- SEVERE: Interrupts a participant's usual daily activities, causes severe discomfort, may cause discontinuation of study drug, and generally requires systemic drug therapy or other treatment.

6.5 Expectedness

For a serious adverse event that is considered possibly related to the study treatment, the Medical Monitor will classify the event as unexpected if the nature, severity, or frequency is inconsistent with the risk information previously described in the background section.

6.6 Coding of Adverse Events

Adverse events will be coded using the MedDRA dictionary. To facilitate coding, the site will enter a preliminary event descriptor code, which the Medical Monitor may accept or change (the Medical Monitor's coding will be used for all reporting and the site's preliminary coding will not be updated or used in reporting).

The Medical Monitor will review the investigator's assessments of causality and severity and may agree or disagree. For causality, the Medical Monitor will only attempt to reconcile differences for causality when one party's coding is no/unlikely and the other party's coding is possible/probable/ definite. For severity, the Medical Monitor will only attempt to reconcile differences when one party codes as severe and the other party as mild/moderate. The investigator's and Medical Monitor's assessments do not need to agree. For reporting purposes, the Medical Monitor will make the final determination with respect to causality as well as whether an event is classified as a serious adverse event. However, both the investigator's and the Medical Monitor's coding will be recorded.

6.7 Outcome of Adverse Event

The outcome of each reportable adverse event will be classified by the investigator as follows:

- RECOVERED/RESOLVED: The participant recovered from the AE/SAE without sequelae. Record the AE/SAE stop date.
- RECOVERED/RESOLVED WITH SEQUELAE: The event persisted and stabilized without change in the event anticipated. Record the AE/SAE stop date.
- FATAL: A fatal outcome is defined as an SAE that results in death. Only the event that was the cause of death should be reported as fatal. AEs/SAEs that were ongoing at the time of death, but were not the cause of death, will be recorded as "resolved" at the time of death. Primary and secondary (if applicable) causes of death will be recorded.

- NOT RECOVERED/NOT RESOLVED (ONGOING): An ongoing AE/SAE is defined as a still ongoing event with an undetermined outcome.
- An unresolved outcome will require follow up by the site to determine the final outcome of the AE/SAE.
  The outcome of an ongoing event at the time of death that was not the cause of death will be updated and recorded as "resolved," with the date of death recorded as the stop date.
- UNKNOWN: An unknown outcome is defined as an inability to contact the participant or access the participant's records to determine the outcome (for example, a participant lost to follow-up).

If any reported adverse events are ongoing when a participant completes the study (or withdraws), adverse events classified as suspected, unexpected serious adverse reactions (SUSARs) will be followed until they are either resolved or have no prospect of improvement or change, even after the participant has completed all study visits/contacts. For all other adverse events, data collection will end when the participant completes the study. Note: participants should continue to receive appropriate medical care for an unresolved adverse event after their participation in the study ends.

6.8 Timing of Event Reporting

Serious or unexpected adverse events must be reported to the JCHR within 24 hours by completing the online adverse event form.

Other reportable adverse events will be reported within three (3) days of the site staff becoming aware of the event by completion of an electronic case report form.

Each principal investigator is responsible for reporting study-related events and abiding by any other reporting requirements specific to his/her local Institutional Review Board or Ethics Committee. As the JCHR IRB is the overseeing IRB, sites must also report all serious adverse events that they have determined to be unexpected and are probably, possibly, or definitely related to the study to the JCHR IRB within seven (7) calendar days.

The JCHR will be responsible for notifying the FDA and all participating investigators of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than seven (7) calendar days after initial receipt of the information. In addition, the JCHR will notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than fifteen (15) calendar days after the sponsor determines that the information qualifies for reporting.

6.9 Safety Oversight

A Data and Safety Monitoring Committee (DSMC) will review compiled safety data at periodic intervals, with a frequency of no less than twice a year. The DSMC can request modifications to the study protocol or suspension or outright stoppage of the study if deemed necessary based on the safety data available. Details regarding the DSMC's review will be documented in a separate DSMC charter.

The objective of the DSMC ongoing review during the study is to decide whether the study (or study treatment for an individual or study cohort) should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group, a particular study site, or the entire study) is a potential outcome of a DSMC safety review.

Chapter 7: Miscellaneous Considerations

7.1 Contacts by the Jaeb Center for Health Research and Sites

The Jaeb Center serves as the PEDIG Coordinating Center. The Jaeb Center will be provided with the parent's contact information. The Jaeb Center may contact the parents of the participants. Permission for such contacts will be included in the Informed Consent Form. The principal purpose of the contacts will be to develop and maintain rapport with the participant and family and to help coordinate the scheduling of the outcome examinations. Parents may or may not opt to receive text/email reminders during the study.

7.2 Participant Compensation

Participant compensation will be specified in the informed consent form.

7.3 Participant Discontinuation of Study Treatment

Omega-3 supplementation must be discontinued if any of the following occur during the study:

- Routine consumption of fish increases to 4 or more times a week (on average).
- Diagnosed with bleeding disorder or begin any anticoagulants.
- Daily use of aspirin, non-steroidal anti-inflammatory (NSAID), or blood-pressure-lowering medications.
- Chemotherapy treatment.
- Use of orlistat (Xenical or Alli).
- Current use of any other medication that the investigator feels may significantly interact with omega-3 supplementation other than oral contraceptives.
- Diagnosis of diabetes mellitus, heart rhythm disorder, underactive thyroid, liver disease, or pancreatic disease.

Cliradex will be discontinued if a participant or parent self-reports pregnancy prior to the 8-week discontinuation of eyelid cleansers.

Additional rules for discontinuing the use of any study treatment are described below:

- The investigator believes it is unsafe for the participant to continue to receive the treatment.
- The parent or participant requests that the treatment be stopped.

Even if the study treatment is discontinued, the participant will be encouraged to remain in the study through the final study visit.

7.4 Participant Withdrawal

Participation in the study is voluntary, and a participant may withdraw anytime. For participants who withdraw, their data will be used up until the time of withdrawal.

7.5 Confidentiality

For security and confidentiality purposes, participants will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the coordinating center, the Jaeb Center for Health Research in Tampa, FL. De-identified participant information may also be provided to research sites involved in the study.

Chapter 8: Statistical Considerations

8.1 Statistical and Analytical Plans

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be finalized before the first enrollment. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

8.2 Statistical Hypotheses

A test of superiority will be used in evaluating the following hypotheses for the primary outcome for each respective active treatment group (**OMEGA-3, ANTI-*DEMODEX***) versus the control group **(STANDARD).** Since two treatments with different mechanisms of action are being compared to a shared control group, no adjustment for multiplicity is necessary (see Section 8.17).

8.2.1 Proportion with Improvement or Resolution of Chalazia at 8 Weeks

8.2.1.1 ANTI-*DEMODEX* vs. STANDARD

- Null Hypothesis (H_0):
  - There is *no difference* between the **ANTI-*DEMODEX*** and **STANDARD** groups in the proportion of participants with improved or resolved chalazia at 8 weeks based on a masked in-office examination at 8 weeks, enrollment in-office exam, and parental photographs taken at enrollment.
- Alternative Hypotheses (H_a):
  - There *is a difference* between the **ANTI-*DEMODEX*** and **STANDARD** groups in the proportion of participants with improved or resolved chalazia at 8 weeks based on a masked in-office examination at 8 weeks, enrollment in-office exam, and parental photographs taken at enrollment.

8.2.2 Proportion with Development of New Chalazia Between 8 Weeks and 12 Months

New-onset chalazia is defined as a new chalazion either occurring in the same area as a previously resolved chalazion or in a new area of the eyelid by all available data.

8.2.2.1 OMEGA-3 vs. STANDARD

- Null Hypothesis (H_0):
  - There is *no difference* between the **OMEGA-3** and **STANDARD** groups in the proportion of participants developing one or more new chalazia (on any eyelid) between 8 weeks and 12 months based on *all available data between these timepoints.*
- Alternative Hypotheses (H_a):
  - There *is a difference* between the **OMEGA-3** and **STANDARD** groups in the proportion of participants developing one or more new chalazia (on any eyelid) between 8 weeks and 12 months based on *all available data between these timepoints*.

Similar hypothesis tests of superiority will be conducted for secondary outcomes. The comparisons will be based on statistics appropriate to the outcome.

8.3 Sample Size

Sample size has not been statistically calculated. Estimates for resolution of chalazia with warm compresses with standard eyelid cleanser (i.e., not anti-*Demodex*) range from 21% to 77% in adults depending on the number of times performed daily and on whether eyelid hygiene was also used,[21-25] but no estimates are available for children; there appear to be no studies that have evaluated warm compresses and anti-*Demodex* eyelid cleanser on resolution of chalazia. Therefore, a convenience sample of 168 (56 participants in each treatment group) will be used. After adjusting for a 10% loss to follow-up at 8 weeks, the total sample size will be 150 (50 per treatment group).

Table 5 shows the half-width of the 95% confidence intervals expected given various observed point estimates for the proportion of participants with improved or resolved chalazia at 8 weeks (or the proportion of participants who developed a new chalazion at any time) in any of the three treatment groups.

This demonstrates the expected precision of the within-group estimates generated in this study.

Note that the half-width is a function of the outcome proportion and is symmetric around 50% (i.e., the half-width for a point estimate of 70% is the same as the half-width for a point estimate of 30%).

Table 5. Half Width of 95% Confidence Interval of Within Treatment Group Proportions (Sample Size per Group = 50)

Outcome Proportion	Half Width of 95% Confidence Interval of Within-Group Estimates[1]
50%	14%
60% or 40%	14%
70% or 30%	13%
80% or 20%	11%
90% or 10%	8%

[1] The half-width of 95% confidence intervals were calculated using normal approximation.

Table 6 shows the half-width of the 95% confidence intervals for differences in the proportion of improved or resolved chalazia at 8 weeks either between the **OMEGA-3** and **STANDARD** Groups or between the **ANTI-*DEMODEX*** and **STANDARD** Groups, given different point estimates of the proportions in the **STANDARD** Group.

Table 7 shows the half-widths for the difference in the proportion of participants with new chalazia at any time. This demonstrates the expected precision of the Omega-3 or the anti-*Demodex* treatment effect.

Table 6. Half-Width of 95% Confidence Interval of Treatment Group Difference in Percentage of Participants with Improved or Resolved in Each Treatment Group (Sample Size per Group = 50)[1]

Difference in Percentage of Participants with Improved or Resolved between Active Treatment Group and STANDARD Group	Percentage of Participants with Improved or Resolved in the STANDARD Group						
	20%	30%	40%	50%	60%	70%	80%
+5%	17%	19%	20%	20%	19%	18%	15%
+10%	17%	19%	20%	20%	19%	17%	14%
+15%	17%	19%	20%	19%	18%	16%	13%
+20%	18%	19%	19%	19%	18%	15%	11%
+25%	18%	19%	19%	19%	17%	14%	----

[1.] The half-widths of 95% confidence intervals were calculated using normal approximation.

Table 7. Half-Width of 95% Confidence Interval of Treatment Group Difference in Percentage of Participants with Improved or Resolved in Each Treatment Group (Sample Size per Group = 50)[1]

Difference in Percentage of New Chalazia at Any Time between Active Treatment Group and STANDARD Group	Percentage of New Chalazia at Any Time in STANDARD Group						
	20%	30%	40%	50%	60%	70%	80%
-5%	15%	18%	19%	20%	20%	19%	17%
-10%	14%	17%	19%	20%	20%	19%	17%
-15%	13%	16%	18%	19%	20%	19%	17%
-20%	11%	15%	18%	19%	19%	19%	18%
-25%	----	14%	17%	19%	19%	19%	18%

[1.] The half-widths of 95% confidence intervals were calculated using normal approximation.

8.4 Outcome Measures

Primary Efficacy Endpoints:

ANTI-*DEMODEX* vs. **STANDARD**

- "Improved or Resolved" chalazia on all eyelids at 8 weeks based on the *masked examiner's office examination at 8 weeks, enrollment in-office exam, and parental photographs taken at enrollment.*

OMEGA-3 vs. **STANDARD**

- One or more new chalazia (on any eyelid) developing between 8 weeks and 12 months based on *all available data between these timepoints.*

Secondary Efficacy Endpoints:

ANTI-*DEMODEX* vs. **STANDARD**

- One or more new chalazia (on any eyelid) developing between 8 weeks and 12 months based on *all available data between these timepoints.*

OMEGA-3 vs. **STANDARD**

- "Improved or Resolved" chalazia on all eyelids at 8 weeks based on the *masked examiner's office examination at 8 weeks, enrollment in-office exam, and parental photographs taken at enrollment.*

ANTI-*DEMODEX* vs **STANDARD** and **OMEGA-3** vs. **STANDARD**

- "Resolved" chalazia on all eyelids based on the *masked examiner's office examination at 8 weeks*.
- Number of new chalazia (on any eyelid) developing between 8 weeks and 12 months based on *all available data between these timepoints.*

Exploratory Efficacy Endpoints:

ANTI-*DEMODEX* vs **STANDARD** and **OMEGA-3** vs. **STANDARD**

- "Improved or Resolved" chalazia on all eyelids based on the masked central reader assessment of *telehealth information (parental photographs, parental palpation of eyelids, and Parental Treatment Outcome Question) at 8-weeks, enrollment in-office exam, and parental photographs taken at enrollment.*
- "Resolved" chalazia on all eyelids based on the masked central reader assessment of *telehealth information (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question) at 8-weeks, enrollment in-office exam, and parental photographs taken at enrollment.*
- "Improved or Resolved" chalazia on all eyelids based on the *masked examiner's office examination, telehealth information (parental photographs, parental palpation of eyelids, Parental Treatment Outcome Question at 8-weeks (primary outcome examination), enrollment in-office exam, and parental photographs taken at enrollment)*
- "Resolved" chalazia on all eyelids based on the *masked examiner's office examination, telehealth information (parental photographs, parental palpation of eyelids, and Parental Treatment Outcome Question at 8-weeks), enrollment in-office exam, and parental photographs taken at enrollment)*.
- Time to development of a new chalazion between 8 weeks and 12 months based on *all available data between these time points.*
- One or more new chalazia developing over the first 8 weeks
- One or more new chalazia developing between baseline and 12 months
- Number of new chalazia developing over the first 8 weeks
- Number of new chalazia developing between baseline and 12 months
- Collarette grading scale at 8 weeks

- Collarette (grade 0) at 8 weeks among participants with greater or equal to grade 2 collarettes (10 or more eyelashes) at enrollment.

Telehealth Endpoints at 8 weeks (all groups combined):

- Agreement for "improved or resolved" chalazia vs NOT "improved or resolved" chalazia Investigator Treatment Outcome Question
  - Investigator Treatment Outcome Question at 8 weeks based on in-office examination *at 8-weeks, enrollment in-office exam, and parental photographs taken at enrollment* ("gold standard")

  versus
  - Central Reader Treatment Outcome Question at 8 weeks based on telehealth information (parental photos, parental palpation of eyelids, Parental Treatment Outcome Question at 8 weeks, enrollment in-office exam, *and parental photographs taken at enrollment*)
- Agreement between for "improved or resolved" chalazia vs NOT "improved or resolved" chalazia.at 8 weeks based on
  - Investigator Treatment Outcome Question at 8 weeks based on in-office examination *at 8-weeks, enrollment in-office exam, and parental photographs taken at enrollment* ("gold standard")

    versus
  - Parental Treatment Outcome Question

Note that the parental 8-week outcome photographs, palpation of eyelids, and Treatment Outcome Question will be considered missing if they are not completed within 2 days before or on the same day before the 8-week primary outcome exam.

8.5 Analysis Datasets and Sensitivity Analyses

Intention-To-Treat (ITT) Analysis Cohort: all randomized participants, irrespective of treatment received, will be analyzed according to treatment assignment.

Safety Analysis Cohort: participants who receive at least one dose of the randomly assigned study treatment (oral omega-3, anti-*Demodex* eyelid cleanser, or standard eyelid cleanser).

The primary analysis will follow the ITT principle. It will include all randomized participants.

The data from the ITT cohort will be analyzed according to the group to which the participants were assigned through randomization, regardless of treatment received.

8.6 Analysis of the Primary Efficacy Endpoints

8.6.1 Improved or Resolved Chalazia on All Eyelids at 8 Weeks

This is the primary outcome for the **ANTI-*DEMODEX*** vs. **STANDARD** comparisons.

The four-level scale from the Treatment Outcome Questionnaire (worse / unchanged / improved / resolved) will be analyzed as a binary outcome defined as "improved or resolved " chalazia vs. not "improved or resolved" chalazia.

The primary outcome will be compared between the two treatment groups using the chi-square test and associated P value. The treatment effect at 8 weeks will be calculated as the risk difference and 95% confidence interval using the Newcombe hybrid score method.[65, 66] Complete case analysis without imputation of missing data will be used. If the expected cell count is less than 5 for any cell, then the Barnard unconditional exact test will be used with the number of participants in each treatment group will be considered as fixed; the 95% confidence interval for risk difference will be calculated using the Agresti-Min mid-P method.[65, 67]

In addition, the proportion of each level of treatment outcome (worse / unchanged / improved / resolved) in each treatment group will be tabulated by group. A sensitivity analysis using the Wilcoxon rank-sum test and Hodges-Lehmann estimator (pseudomedian) will be performed to compare the ordinal treatment outcome between groups, which may be more powerful than comparing a dichotomized outcome.

8.6.2 New Chalazia Developed on Any Eyelid Between 8 Weeks and 12 Months

This is the primary outcome for the **OMEGA-3** vs. **STANDARD** comparisons.

A new chalazia refers to chalazia that develops at the location of the original chalazia and chalazia that develops at a different location. All new chalazia will be included regardless of whether detected on clinical exams, detected on parental photographs, or reported by the parent but not substantiated on clinical exam or on parental photographs. Chalazia first noted at the 8-week visit or reported by the parent at one-month will not be counted for this outcome, but chalazia noted at the 6 month or 12-month visit or reported by the parent any time after 8 weeks will be counted. For participants who do not complete the 8-week visit, any chalazia occurring after the target date of the visit will be counted. Participants who have new chalazia during the first 8 weeks will be included in this analysis but the chalazia that developed during the first 8 weeks will not count as outcomes.

The cumulative proportion of participants developing one or more new chalazia on any eyelid between 8 weeks and 12 months will be obtained using the Kaplan-Meier method and compared between the treatment groups using the Z test. The estimates of cumulative proportions and their standard errors from the Kaplan-Meier method will be used to calculate the treatment group difference of proportions of developing one or more new chalazia between 8 weeks and 12 months. The 95% confidence interval of the treatment group difference will be calculated based on the Z test. Data from participants lost to follow-up or who complete all visits but do not develop chalazia will be censored at the participant's final completed visit. To aid in interpretation, a Kaplan-Meier plot showing time to new chalazia development by treatment group will be created. The cumulative number of events and censored participants will be tabulated by treatment group.

8.7 Analysis of the Secondary Efficacy Endpoints

Note that for the primary outcomes above, the same methods will be used to analyze the same endpoints as secondary outcomes for comparisons between **STANDARD** and the group for which the outcome was not primary (**ANTI-*DEMODEX*** or **OMEGA-3**).

8.7.1 Resolved Chalazia on All Eyelids at 8 Weeks

The four-level scale from the Treatment Outcome Questionnaire (worse / unchanged / improved / resolved) will be analyzed as a binary outcome defined as “resolved" chalazia vs. not “resolved” chalazia. Analysis will otherwise mimic the primary outcome.

8.7.2 Number of New Chalazia on Any Eyelid Between 8 weeks and 12 Months

The number of new chalazia developed between 8 weeks and 12 months will be compared between the treatment groups using the Wilcoxon rank-sum test. The between-group differences will be summarized using the Hodges Lehmann estimator and 95% confidence interval.

8.8 Analysis of the Exploratory Efficacy Endpoints

The exploratory efficacy endpoints of “Improved or Resolved” and “Resolved” chalazia are all binary outcomes and will be analyzed using the same approach planned for the primary outcome (Section 8.4).

Time to development of a new chalazion between 8 weeks and 12 months based on all available data between these time points will be compared using the log-rank test.

The number of new chalazia developing over the first 8 weeks and 12 months will be tabulated and compared between groups using the Wilcoxon rank-sum test. The between-group differences will be summarized using the Hodges Lehmann estimator (pseudomedian) and 95% confidence interval.

The proportion of participants with one or more new chalazia developing over the first 8 weeks and 12 months will be compared between the groups using a Z test similar to Section 8.6.2.

The collarette grading scale at 8 weeks is an ordinal outcome and will be compared between groups using the Wilcoxon Rank Sum Test. The between-group differences will be summarized using the Hodges Lehmann estimator and 95% confidence interval.

The proportion of participants with collarette grade 0 at 8 weeks among participants with greater than or equal to grade 2 collarettes at enrollment is a binary outcome and will be analyzed using the chi-square test.

8.9 Analysis of the Telehealth Endpoints at 8 Weeks

The participants from all three treatment groups will be combined to analyze the telehealth endpoints. Agreement between each method will be calculated as sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals (Wilson method). Missing data will not be imputed.

8.10 Additional Tabulations

The following assessments will be tabulated according to the treatment group.

- Clinical assessment of chalazia based on the slit lamp examination by the masked examiner at 8 weeks, 6 months, and 12 months.
- Proportion of screened participants (had signed consent; in office test complete) who decline to participate in the RCT because will no longer accept possible randomization to

Cliradex and/or omega-3.
- Treatments prescribed at investigator discretion between 8 weeks and 12 months.
- Proportion of participants who received surgery between 8 weeks and 12 months.

8.11 Safety Analyses

All reported adverse events will be tabulated by the treatment group.

8.12 Intervention Adherence

Adherence to study treatment (oral omega-3, anti-*Demodex* eyelid cleanser, and standard eyelid cleanser) and adherence to warm compresses will be assessed using calendars brought to the 6-, and 12-month outcome exams and tabulated in each treatment group.

8.13 Protocol Adherence and Retention

Protocol deviations and visit completion rates (excluding participant deaths) will be tabulated for each treatment group.

8.14 Baseline Descriptive Statistics

The following baseline characteristics will be tabulated according to treatment group:

- Demographic information (age, sex, race, ethnicity)
- Treatment history of current and any prior chalazia
- Clinical assessment of chalazia

8.15 Planned Interim Analyses

There are no formal planned interim analyses for this phase II randomized trial. The Data and Safety Monitoring Committee will review safety and efficacy data approximately every 6 months and have the authority to stop the trial if necessary.

8.16 Subgroup Analyses

Subgroup analyses will be considered exploratory given anticipated small sample size and low statistical power. Therefore, results will be interpreted cautiously, particularly if the primary analysis does not indicate a treatment benefit. A minimum of 20 participants per subgroup in each treatment group will be required to conduct a formal subgroup analysis; otherwise, outcomes will be tabulated by subgroup without formal comparison. Cut points for continuous variables will be specified in the SAP. The following baseline factors will be evaluated in subgroup analyses:

- Age
- Inflammation of any chalazia (yes vs no)
- Erythema of any chalazia (none vs mild/moderate/severe)
- Duration of current chalazia
- Size of largest chalazion
- Number of chalazia
- Number of eyelids affected
- Previous chalazia in the past year (none, one, multiple)
- Dosage of oral omega-3 (1 g or 2 g)
- Grading of collarettes

- Presence of Meibomian gland disorder (plugging of Meibomian gland or grade 1 or higher on Meibomian gland expression scale)

Prior research neither supports nor negates significant differences in treatment effects by sex and race and/or ethnicity. Therefore, consistent with NIH Notice Number NOT-OD-25-131, exploratory subgroup analyses will also be conducted by sex and race and/or ethnicity.

8.16.1 Improved or Resolved Chalazia on All Eyelids at 8 Weeks

The treatment group difference in the percentage of "improved or resolved" chalazia versus not "improved or resolved" at 8 weeks based on the masked examiner's office examination will be explored using binomial regression with fixed effects for treatment group, the baseline factor, and their interaction. Within-subgroup risk differences and 95% confidence intervals will be presented as a forest plot.

8.16.2 New Chalazia Developed on Any Eyelid Between 8 Weeks and 12 Months

The treatment group difference in cumulative proportions of participants developing one or more new chalazia on any eyelid between 8 weeks and 12 months will be explored by testing for an interaction as the difference of within-subgroup treatment group differences in Kaplan-Meier cumulative probabilities.. Within-subgroup differences in cumulative proportions and 95% confidence intervals will be presented as a forest plot.

8.17 Multiple Comparisons/Multiplicity

This study has two main sources of multiplicity: multiple endpoints (Section 8.4) and multiple treatment groups compared with a shared control group (Section 8.2). The fixed sequence method will be used to control for multiplicity arising from multiple endpoints.[68] Each primary and secondary efficacy outcome will be evaluated in the prespecified order below. Note that the testing order differs for the comparisons of **ANTI-*DEMODEX*** vs **STANDARD** and **OMEGA-3** vs **STANDARD**. If the null hypothesis of no difference is rejected for the first outcome, the next outcome in the sequence will be tested without further adjustment. If a null hypothesis is not rejected, all subsequent tests will be considered exploratory, and P values will not be reported. The treatment group differences and the 95% confidence intervals will be calculated. The fixed sequence method will provide strong control of the familywise error rate.

Table 4. Fixed Sequence of Primary and Secondary Outcomes by Comparison

Order	ANTI-*DEMODEX* vs STANDARD	OMEGA-3 vs STANDARD
1	"Improved or Resolved" chalazia on all eyelids based on the masked examiner's office examination at 8 weeks, *enrollment in-office exam, and parental photographs taken at enrollment*.	One or more new chalazia (on any eyelid) developing between 8 weeks and 12 months based on *all available data between these timepoints*
2	"Resolved" chalazia on all eyelids based on the *masked examiner's office examination at 8 weeks*, *enrollment in-office exam, and parental photographs taken at enrollment*.	Number of new chalazia (on any eyelid) developing between 8 weeks and 12 months based on all available data between these timepoints.

3	One or more new chalazia between 8 weeks and 12 months based on *all available data between these timepoints*	"Improved or Resolved" chalazia on all eyelids based on the masked examiner's office examination at 8 weeks.
4	Number of new chalazia (on any eyelid) developing between 8 weeks and 12 months based on all available data between these timepoints.	"Resolved" chalazia on all eyelids based on the *masked examiner's office examination at 8 weeks*.

Exploratory comparisons, including subgroup analyses, will be considered hypothesis-generating and will require confirmation in future studies. There will be no adjustment for multiplicity for exploratory outcomes. Nominal 95% CIs will be reported without P values.

Although two pairwise comparisons are being evaluated for each outcome (**OMEGA-3** vs **STANDARD** and **ANTI-*DEMODEX*** vs **STANDARD**), there will be no formal adjustment to the familywise error rate due to multiple treatment group comparisons. Because the primary objective of this trial is to compare two active treatments (oral omega-3 and anti-*Demodex* eyelid scrub) that have different mechanisms of action with a shared control group (and not with one another), a multiplicity adjustment is not needed.[69-71] The risk of a false positive finding with this approach is lower than if the two hypotheses were evaluated in two studies with different control groups. The same logic applies to secondary, exploratory, safety, and subgroup analyses.

8.18 Exploratory Analyses Comparing ANTI-*DEMODEX* vs OMEGA-3

Exploratory comparisons between **ANTI-*DEMODEX*** and **OMEGA-3** will be conducted for all primary, secondary, and exploratory efficacy outcomes evaluated for the **ANTI-*DEMODEX*** vs **STANDARD** and **OMEGA-3** vs **STANDARD** comparisons. Analyses methods will be identical to those used for the **ANTI-*DEMODEX*** vs **STANDARD** and **OMEGA-3** vs **STANDARD** comparisons.

Chapter 9: Data Collection and Monitoring

9.1 Case Report Forms and Other Data Collection

Study data are collected on electronic case report forms (CRFs). When data are directly collected in electronic case report forms, this will be considered the source data. For any data where the eCRF is not the source (e.g., lab results transcribed from a printed report into the eCRF), the source documentation must be retained in the participant's study chart or medical record. This source must be readily verifiable against the values entered into eCRF. Even where all study data are directly entered into the eCRFs at office visits, evidence of interaction with a participant must be recorded (e.g., office note, visit record, etc.)

Each participating site will maintain appropriate medical and research records for this trial in compliance with ICH E6 and regulatory and institutional requirements for the protection of participants' confidentiality.

9.2 Study Records Retention

Study documents should be retained for a minimum of 3 years after completion of the final grant reporting. These documents should be retained for a longer period, however, if required by local regulations.

9.3 Quality Assurance and Monitoring

Designated personnel from the Coordinating Center will be responsible for maintaining quality assurance (QA) and quality control (QC) systems. These systems ensure that the clinical trial is conducted and that the data is generated, documented, and reported in accordance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements. Additionally, they will ensure that the rights and well-being of trial participants are protected and that the reported trial data are accurate, complete, and verifiable. Adverse events will be prioritized for monitoring.

A risk-based monitoring (RBM) plan will be developed and revised as needed during the study, consistent with the FDA's "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013). This plan describes who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

The data of most importance for monitoring at the site are participant eligibility and adverse events. Therefore, the RBM plan will focus on these areas. As much as possible, remote monitoring will be performed in real-time with on-site monitoring to evaluate the verity and completeness of the key site data.

Elements of the RBM may include:

- Qualification assessment, training, and certification for sites and site personnel
- Oversight of Institutional Review Board (IRB) coverage and informed consent procedures
- Central (remote) data monitoring: validation of data entry, data edits/audit trail, protocol review of entered data and edits, statistical monitoring, study closeout

- On-site monitoring (site visits): source data verification, site visit report
- Study drug accountability
- Communications with site staff
- Participant retention and visit completion
- Quality control reports
- Management of noncompliance
- Documenting monitoring activities
- Adverse event reporting and monitoring

Coordinating Center representatives or their designees may visit the study facilities at any time to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation, and discussion of the conduct and progress of the study.

The investigational site will provide direct access to all trial-related sites, source data/documents, and reports for monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

9.4 Protocol Deviations

A protocol deviation is noncompliance with the clinical trial protocol, GCP, or procedure requirements. Noncompliance may be on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

The site PI/study staff are responsible for knowing and adhering to their IRB requirements. Further details about handling protocol deviations will be included in the monitoring plan.

Chapter 10: Ethics/Protection of Human Participants

10.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Participants of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

10.2 Institutional Review Boards

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. The protocol, consent, and assent forms must be approved before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

10.3 Informed Consent Process

10.3.1 Consent Procedures and Documentation

Informed consent is a process that is initiated before the individual agrees to participate in the study and continues throughout the individual's study participation. Extensive discussion of the risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be approved by the IRB, and the participants will be asked to read and review the document. The investigator will explain the study to the participant and answer any questions. All participants will receive a verbal explanation of the study's purposes, procedures, potential risks, and their rights as research participants, using language appropriate to their level of understanding. Participants will be able to carefully review the written consent form and ask questions before signing.

The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document before any procedures are done specifically for the study. The participants may withdraw their consent at any time throughout the trial. The participants will be given a copy of the informed consent document for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.4 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies, or company supplying the study product may inspect all documents and.

cords required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will remain secure for as long as dictated by the reviewing IRB, institutional policies, or sponsor requirements.

Study participant research data for statistical analysis and scientific reporting will be transmitted to and stored at the Jaeb Center for Health Research. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and management systems used by clinical sites and Jaeb Center for Health Research staff will be secured and password protected.

At the end of the study, all study databases will be de-identified and archived at the Jaeb Center for Health Research.

To further protect the privacy of study participants, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others with access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.5 Future Use of Data

Data collected for this study will be analyzed and stored at the Jaeb Center for Health Research. After the study is completed, the de-identified, archived data will be made available to the public.

Chapter 11: References

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